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THE ANNALS OF APPLIED BIOLOGY

EDITED BY

W. B. BRIERLEY

AND

D. WARD CUTLER

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BY

W. B. BRIERLEY

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STUDIES ON POTATO VIRUS DISEASES

VIII. ON A RINGSPOT VIRUS AFFECTING SOLANACEOUS PLANTS

BY KENNETH M. SMITH, D.Sc., Ph.D.

(*Potato Virus Research Station, School of Agriculture, Cambridge.*)

(With Plates I–V.)

CONTENTS.

I. INTRODUCTION.

THE virus disease described in this communication was first noticed in 1929 in a commercial glasshouse in Cardiff, affecting the solanaceous plant *Solanum capsicastrum*, and, so far as the writer is aware, it is the first record of the disease in the British Isles. The investigation of this ringspot virus was undertaken chiefly in the hope that a study of its reactions in the Solanaceae might assist in the study of the mosaics, crinkles and streaks of the potato plant and the ringspot diseases they cause in tobacco (3, 5). Previous to the discovery of this virus in *S. capsicastrum*, the writer had been of the opinion that ringspot of tobacco as it occurs in America and elsewhere might be due to a potato virus such as mosaic or streak. Now, however, owing to the extraordinary similarity of certain phases in the symptom expression of the respective viruses in tobacco, it seems evident that the true ringspot of tobacco has no connection with the potato mosaic group of viruses. Moreover, it would appear that the tobacco ringspot described by Priode (2), Wingard (7) and others, is again a different virus in spite of the close similarity of the leaf symptoms. These workers were able to infect plants of widely differing orders with their ringspot, while the ringspot described in the present paper appears to be confined to the Solanaceae, the latter also

usually produces a severe necrosis of the stem and leaf veins of the tobacco plant and occasionally concentric rings on the stem, while Priode (2) found that his ringspot attacked leaf-tissue only, and that no visible symptoms ever appeared on stems or large leaf veins. It seems, indeed, likely that "ringspot" is an alternative symptom expression for many mosaic viruses. The development of the "ringspot" symptoms probably depends upon the degree of virulence of the virus, the type of host plant and possibly to some extent upon the method of infection of that host plant.

The present work consists of a comparative study of the symptoms produced by the ringspot virus upon a number of solanaceous plant hosts, including the potato, and of the methods of transmission of the virus with some preliminary investigation of the insect vectors.

The following Solanaceae were used in the infection experiments:

S. capsicastrum.
S. laciniatum.
S. tuberosum (potato).
S. nodiflorum.
S. nigrum (black nightshade).
Lycopersicum (tomato).
Nicotiana tabacum (tobacco, vars. White Burley and Virginia).
N. glauca.
Datura stramonium (thorn apple; jimson weed).
Capsicum spp.

The writer is greatly indebted to Mr J. Rees, advisory mycologist at the University College of South Wales, Cardiff, who gave him the original plant of *S. capsicastrum* affected with the ringspot virus. Acknowledgment is also due to Miss M. E. Sewell for her care of the plants used in these experiments, and to the editor of *Discovery* for permission to reproduce Plate I, fig. 2.

II. A COMPARATIVE STUDY OF THE REACTIONS OF VARIOUS SOLANACEAE TO THE RINGSPOT VIRUS.

Solanum capsicastrum.

Description of symptoms.

As already mentioned, the ringspot virus was first discovered affecting *S. capsicastrum*, and one such diseased plant of this species was the sole source of all the virus used in the experiments. It is upon *S. capsicastrum* that the ringspot type of symptom is best expressed. The first sign of infection is the development of numbers of faint spots on the younger,

but not necessarily the youngest, leaves. These faint spots then take on a ring-like form, which may consist of as many as six concentric rings with a central spot. Occasionally neighbouring groups of rings become linked together, or diamond-shaped patterns form across the veins, the whole effect being most striking (Plate I, figs. 1, 2). As the leaves become older, necrosis sets in between the rings, giving the leaf a whitish appearance (Plate I, fig. 2). No type of symptom other than that of the rings and diamonds has been observed with this virus upon *S. capsicastrum*, though occasionally there occur scroll-like or wavy lines which fail to form any distinct pattern.

Transmission of the ringspot virus to S. capsicastrum.

(*a*) *By grafting.* As is probably the case with all plant viruses it is possible to disseminate the virus by this means. Healthy stocks of *S. capsicastrum*, grafted with scions of the ringspot plant on July 25th, showed first signs of infection on September 10th, 47 days later. The rings appeared in the shoots nearest to the graft and the virus only spread very slowly through the rest of the plant; in some cases symptoms were confined to the shoot nearest to the graft. From observations made on a number of infection experiments it appears that this ringspot virus moves very slowly through *S. capsicastrum*.

(*b*) *By needle scratch.* A large number of healthy seedlings of *S. capsicastrum* were inoculated by needle scratch with the ringspot virus. The number of positive inoculations was, however, very small, not more than about 5–10 per cent. of the inoculated plants becoming infected. Symptoms developed in 6 to 8 weeks; in some cases the symptoms developed normally, the infection being obviously systemic, while in others one isolated ring only appeared on a leaf far removed from the point of inoculation, the remainder of the plant being quite normal in appearance and showing no other symptoms whatever. It is difficult to say in this latter case whether infection was systemic or was confined to the leaf showing the isolated ring, a condition similar to that described by Henderson Smith (1), because in any case positive needle inoculation, even from obviously diseased plants, was so often negative. This state of affairs must be differentiated from that sometimes occurring in needle transmission of the same virus to tobacco (p. 6), where rings appear on the *inoculated* leaf but no further development occurs and the disease dies out without becoming systemic.

(*c*) *Insect transmission.* Attempts were made to induce two species of aphides to disseminate the ringspot virus from diseased to healthy

S. capsicastrum. The insects used were *Myzus persicae* Sulz. and *Macrosiphum gei* Koch. Negative results were obtained with *M. gei* while, with *M. persicae*, out of a large number of experiments, one doubtful case of infection was obtained. In this instance a few wavy lines appeared on one leaf only but no general infection developed. That *M. persicae* can transmit this virus, however, to certain solanaceous plants is shown by the experiments with *Datura stramonium* and *Capsicum*. As the *S. capsicastrum* was presumably infected in the first instance by insect agency, there probably exists another more efficient vector of the virus to this particular plant.

Nicotiana tabacum (White Burley and Virginia).

The ringspot virus was transmitted to tobacco by needle inoculation only; attempts to transmit the virus by aphis (*M. persicae*) failed, while no grafting experiments were made.

As the reactions of the tobacco plant to the ringspot virus give rise to several points of interest the symptoms which are varied in their expression are described in some detail. In the first place a number of White Burley and Virginia tobacco plants were inoculated by needle scratch with sap from the ringspot *S. capsicastrum* shown in Plate I, figs 1, 2. Out of twelve plants, six of each variety inoculated on June 27th, two White Burley plants developed symptoms on July 7th, 10 days later; a very much shorter incubation period than that required by *S. capsicastrum*. The first sign of infection was the appearance of two sets of concentric rings along the inoculation scratches, one set of rings being in close connection with the veins of the leaf (Plate II, fig. 2). Later, the rings increased greatly in number and decreased in size appearing all over the surface of the leaf in the manner shown in Plate III, fig. 2. Thus far the symptom expression is often indistinguishable from the ringspot produced in tobacco by needle inoculation with certain potato viruses, with the possible exception of a greater tendency on the part of the ringspot from *S. capsicastrum* to attack the veins (see Plate III, figs. 1, 2). From a tobacco plant thus infected with the ringspot it is easy to transmit the virus to other tobacco plants by needle scratch. If the tobacco plant is not killed by the virus at the outset as sometimes happens, the symptoms undergo a change with the continued growth of the plant. The ring element in the leaf is less evident and a severe necrosis of the veins and stem of the plant develops (Plate III, figs. 3, 4). Occasionally, however, concentric rings occur upon the main stem until they are lost in the general necrosis.

Although it cannot be said definitely that progressive inoculation increases the virulence of this ringspot virus, yet it is clear that the degree of virulence may vary greatly, the rings being associated with the less virulent form. Another type of symptom exhibited by this virus in the tobacco plant is of a much greater virulence, and here the ringing is usually suppressed or absent; this most virulent form may be called "scorch" rather than "ringspot" of tobacco. This type is fatal to about 50 per cent. of the seedlings in which it develops, and the conditions governing the development of this virulence are at present obscure, though it may, in part, be due to high temperatures. The first sign of the disease is a "clearing of the veins" of the younger leaves, the veins becoming yellow and strongly outlined; from this stage the progress of the disease is very rapid. The leaves quickly lose all green colour and turn dark grey to almost black, rather as if they had been scorched, and often assume a twisted and distorted appearance. In some cases the distortion is such that the leaf is reversed so that the undersurface is presented uppermost. Occasionally the "scorch" is confined to one side of the midrib of the leaf, the other side remaining green. At this point the plant either dies completely or dies down except for the central shoot. In the latter case, growth is resumed after a period, and an apparently healthy plant is produced which may grow normally for as long as 4 to 5 weeks, but after the lapse of this period violent symptoms re-develop and the plant collapses again. This may be repeated two or three times. A similar phenomenon has been described by the writer in inoculation studies with the most virulent type of ringspot produced by a potato virus on tobacco (5). A third type of symptom expression occurs in older plants and resembles that of leaf-drop streak in potato. It appears to be a later stage of the "scorch" which has not proved fatal to the young plant. In this "streak" the chief symptoms are the continual killing out of the growing points of the plant as growth proceeds, together with brown lesions on such leaves as survive and sometimes concentric rings on the stem. The growth of such a plant represents a continual struggle against the virus, each new shoot being destroyed as it is formed. The final result is a tall stem 2 to 3 feet high with numbers of dead shoots clustered upon it, culminating in an apical shoot which, though necrotic, usually survives and remains partially green and may, on occasion, produce flowers and seed (Plate IV, fig. 1).

In connection with the "streak" type of symptom, it happened in a single case that one of the new shoots formed did not succumb to the virus but continued to grow, green and symptomless and apparently free

of, or immune to, the virus, while above and below it the sister shoots continued to die out. The plant in question is figured in Plate IV, fig. 2. This plant produced two heads of flowers, each of which set seed, one on the end of the diseased parent stem and one on the green and apparently virus-free portion. Seed was saved from these two sets of flowers, and the plants arising from them were inoculated under identical conditions with the ringspot virus, in the hope that the plants from the seed produced by the apparently immune half of the plant might prove to be resistant to the virus. There was, however, no difference in the reaction of the two sets of seedlings to the ringspot virus, both batches of inoculated plants developing the disease in due course.

The only other type of reaction of the tobacco plant to the ringspot virus observed was the occasional production of local symptoms only. In such cases a few rings or round necrotic spots appeared upon the inoculated leaves, but no systemic infection developed and the plant continued to grow normally, the local symptoms finally disappearing.

Seed saved from tobacco plants affected with the ringspot virus always produced healthy plants. Positive cross-inoculations by needle were carried out from ringspot tobacco to *S. capsicastrum* and *D. stramonium*. Inoculations from ringspot tobacco to healthy potato were consistently negative.

Datura stramonium (thorn apple; jimson weed).

This plant was as a rule comparatively easy to infect, symptoms developing in 8 to 10 days. Needle inoculation proved the easiest method of dissemination of the ringspot virus to *Datura*, though insect transmission experiments were also carried out with positive results, and will be described later. There were three main types of symptoms produced which appeared to vary according to the virulence of the virus strain being used. These may be described as (*a*) ringspot, (*b*) mosaic, (*c*) crinkle. The ringspot form appeared only to develop after inoculation from ringspot *S. capsicastrum* and not after cross-inoculation between *Daturas*, and consisted of numbers of small double concentric rings with necrotic walls. As a rule the rings did not long persist, but their place was taken by a typical mosaic consisting of a bold mottling of light green or yellow upon a dark green background, being in fact almost indistinguishable from the symptoms produced by needle inoculation of *Datura* with various potato viruses of the mosaic-crinkle-streak group. Occasionally a type of symptom developed which appeared to be intermediate between "mosaic" and "crinkle." In this form the surface of the leaf was puckered,

with the edges turned downwards and inwards, while the leaf assumed a greyish mottled appearance, giving in a marked degree a curious "snakeskin" effect (Plate IV, fig. 3).

The third or "crinkle" type of symptom in *Datura* was usually produced by needle inoculation from a tobacco plant affected with the "scorch" type of symptom produced by the virus in its most virulent form. In "crinkle" the leaf was considerably distorted, the veins were necrotic and yellow, while the interveinal tissue appeared to stand out above the normal level and presented a bold mottling of yellow and dark green. This more acute form of symptom persisted only in the young plant, the whole tendency being for the virulence to decrease rapidly with the ageing plant, and for the crinkle to give way to the mosaic mottling. Cross-inoculation by needle was effected between *Datura* and tobacco and *Datura* and *Capsicum*.

Insect transmission of the ringspot virus to Datura stramonium.

Successful transmission of the ringspot was effected between diseased and healthy *Datura* by means of the aphis *Myzus persicae*, symptoms of mosaic, not ringspot, developing after 9 to 10 days. Needle inoculation of tobacco from an aphis-infected *Datura* reproduced the disease in tobacco in the ordinary severe form showing that passage of the virus through the aphis had not decreased its virulence. The percentage of infection attained by means of *M. persicae* was not very high, and it is possible that a more efficient vector exists. Negative results were obtained in preliminary experiments with the aphis *Macrosiphum gei* and *Thrips tabaci*. Seed from infected *Datura stramonium* gave rise to healthy plants.

SOLANUM TUBEROSUM (potato).

The behaviour of this ringspot virus upon the potato is of considerable interest. Three methods of transmission were used in the experiments, but only one was successful. It was found impossible to infect the potato plant by needle inoculation, at all events under the writer's conditions, however virulent the virus might be. Infection experiments with the aphides *Myzus persicae* and *Macrosiphum gei* also gave negative results. The virus could, however, be disseminated to the potato by grafting, and the most suitable plant for this purpose was found to be *Datura stramonium*. Scions of *Datura* affected with the ringspot disease in its mosaic expression were grafted on to healthy potatoes, Arran Victory and President. Symptoms developed after periods of 25 to 28 days.

Leaf symptoms.

The first sign of infection is the appearance of pale round spots upon the younger leaves; a red necrotic border then develops round the spot, giving it a ring-like appearance (Plate V, fig. 2). In the potato the rings do not attain to such clear-cut perfection of outline as they do on certain others of the Solanaceae. As the disease develops the rings degenerate into necrotic spots, and finally irregular lesions develop in the leaves, the symptom expression in this stage being remarkably reminiscent of some forms of potato streak (Plate V, fig. 3). Needle inoculation from such infected potatoes to tobacco plants produced in the latter the normal ringspot with stem necrosis, the virus did not appear to have altered in any way by passage through the potato. It is of interest to find that, although it was apparently impossible to *inoculate* potato with the ringspot virus from tobacco affected with the disease, yet it was quite easy to inoculate tobacco from potato affected with the same ringspot.

Tuber symptoms.

Unlike many viruses which affect the potato plant, the ringspot under discussion produced characteristic and unmistakable tuber symptoms. When the President and Arran Victory potato plants, grafted as above described, were harvested in the autumn of 1929, the tubers were found to be hard and woody with a typical indurated exterior. On cutting such a tuber across, the interior presented a clear glassy appearance, the whole being rather reminiscent of the hard unrotted parent sett sometimes associated with leaf-roll plants. These tubers, about twelve in all, were stored away and re-examined in February 1930, when it appeared that some change had taken place in the starch content. Needle inoculation into tobacco from one of these tubers produced the normal ringspot disease, showing that the virus was still viable after a winter spent in the resting tuber. A number of the hard tubers were then planted and grown in the insect-proof glasshouse. It was found that sprouting was delayed as compared with healthy tubers of the same varieties, but, once started, growth appeared to be normal. All these tubers gave rise to apparently healthy plants with no symptoms, except for a faint mottling, which may or may not have been connected with the ringspot. The parent tuber remained unrotted in the soil, and it was possible by abstracting the tuber from the pot to produce a number of plants from the same uncut potato. One small indurated tuber of President produced in this manner three large apparently normal plants without any cutting or mutilation, the hard sett producing fresh shoots after each replanting, although the tuber

itself was quite devoid of starch. Sometimes after the production of three or more plants the hard tuber commenced to break down. This production of a number of plants from one tuber is also possible with a normal potato, but appears to be accentuated in this case, probably because of the increased durability of the hard tuber. Core-grafting of normal tubers from one of these indurated potatoes revealed the fact that the tuber symptoms were extremely infectious. A small core from an affected potato inserted in a normal tuber was found to induce the same hardness in the latter within a few weeks. Such a core-grafted tuber, when grown, produced an apparently normal plant, the hardened parent sett behaving similarly to the original affected tuber from which it had been core-grafted. A similar effect could sometimes be produced in a normal tuber by inserting a plug of tobacco or *Datura* leaf affected with the ringspot virus. On harvesting the plants the tubers appeared to be quite normal although the parent setts, including those plugged with infected cores, were still hard and unrotted in the soil. The only explanation of this phenomenon appears to be as follows. It is possible to infect the potato plant with the ringspot virus by grafting, and the virus descends normally into the tuber. It is also possible to infect the tuber by core-grafting with infected tuber material, but the virus does not ascend into the plant and in consequence the new tubers are virus-free. This explanation is only given subject to consequent correction after further study of the problem. The following experiments were performed in this connection: tubers of leaf-roll British Queen and crinkle Myatt's Ashleaf were core-grafted with plugs of infected tuber material. This was done on the supposition that, as the viruses were bound to mix inside the tuber, the leaf-roll or crinkle virus as it ascended the stem would conceivably draw with it the ringspot virus, symptoms of which would then develop in the leaves. The result, however, was that although the core-grafted crinkle and leaf-roll tubers became hard, the plants produced exhibited only crinkle and leaf-roll without any indication of the ringspot disease.

CAPSICUM spp.

The virus was transmitted to this plant both by needle inoculation and by the aphis *Myzus persicae*. In both cases the source of inoculum was an infected *D. stramonium*. Capsicum plants inoculated by needle scratch developed symptoms after about 21 days; the first symptoms took the form of a general pallor of the leaf with a small number of rings; later a marked yellow and green mosaic mottling developed (Plate IV, fig. 4). Inoculation by means of *M. persicae* also produced a number of quite

typical rings, symptoms developing as a rule in 3 to 4 weeks, though in one case development of symptoms was delayed from March 18th to May 3rd, a period of 45 days. This production of the normal ring type of symptom in *Capsicum* by the agency of the aphis *M. persicae* is a point of difference from the other ringspot induced by needle inoculation of tobacco with potato viruses. In this latter case the writer has shown (3, 5) that *M. persicae* will not transmit this disease, and here the ring type of symptom may possibly be connected with the needle scratch method of inoculation.

Lycopersicum (tomato).

The tomato, as was the case with the potato, could be infected by grafting only; needle and aphis inoculation both failed to take under the writer's conditions. Grafting with infected *Datura* produced a mosaic mottling of dark and light green, accompanied by some malformation of the younger leaves which tended to become folded under and almost tubular in appearance.

Solanum laciniatum.

Needle inoculation into this plant from infected *Datura* produced symptoms of disease in 25 days. The symptom expression in this species was quite distinctive, first signs of the disease appeared as a "clearing of the veins" of the youngest leaves accompanied by small necrotic spots. Later, large *single* rings of a characteristic purple tinge developed on the older leaves (Plate V, fig. 1). The growing point of the plant was somewhat twisted and distorted.

Nicotiana glauca.

This species proved somewhat resistant to the ringspot virus; needle inoculation produced, in a few cases, faint rings which disappeared with continued growth of the plant. There was no necrosis.

Solanum nigrum (black nightshade).

This plant could be infected by needle scratch from ringspot tobacco (White Burley). Symptoms developed in 15 days and took the form of clear double or treble concentric rings. *S. nigrum* is less easy to infect than some other members of the Solanaceae, and the percentage of positive inoculations remained low.

Solanum nodiflorum.

Symptoms developed in *S. nodiflorum* 13 days after needle inoculation with the ringspot virus. They took the form of numbers of small necrotic

rings which later lost their individuality and coalesced into an irregular formation. Afterwards there followed considerable distortion and general necrosis of the leaves.

III. PLANTS INOCULATED, OTHER THAN THE SOLANACEAE.

The following plant species belonging to other orders than the Solanaceae were inoculated with the ringspot virus with negative results. Many of these plants were inoculated by Wingard (7) with his ringspot virus with positive results.

Phytolacca decandra L. (pokeweed). *Antirrhinum* spp. (snapdragon). *Tetragonia expansa* Murr. (New Zealand spinach). Asters. French beans. Cotton. Spinach. Beet. Sugar-beet. Cruciferae (various).

IV. DISCUSSION.

Perhaps the most outstanding fact revealed by the study of this ringspot virus is the wide range of difference in its symptom expression upon different solanaceous plants. Owing to this variety of symptoms it could easily be taken for a number of viruses. For example, on *Datura* the virus appears in the form of a mosaic or crinkle which is transmissible both by the aphis *M. persicae* and by needle scratch; while upon potato it takes a streak-like form, apparently transmissible only by grafting. There is, however, no reason to suppose that under standardised conditions of environment the same types of symptoms would not always recur upon the same host plants.

In considering these different effects of the ringspot virus upon the various solanaceous plant hosts, many points of similarity in its behaviour to that of the potato mosaic group may be noticed. So far as could be ascertained, the ringspot is a single entity only and not a complex of viruses, yet it shows many of the characteristics of the potato virus group, mosaic-crinkle-streak, which are at the moment considered separate viruses. The virus now under discussion assumes the ringspot form on certain plant hosts; so also do several of the potato mosaic group. Again, what may be crinkle or mosaic in certain potato varieties appears as streak upon others; similarly, the *capsicastrum* ringspot commonly assumes a streak-like form upon both potato and tobacco. It is thought that potato crinkle, and possibly potato streak also, can appear in a mosaic-like form, so in the same way it is possible for the *capsicastrum* ringspot to appear as a severe crinkle or a mild mosaic according to the degree of virulence and species of host plant. Too much significance should not perhaps be attached to these similarities of symptom ex-

pression, nevertheless such a wide variation in symptoms, exhibited by a single virus, does seem to offer some evidence that the number of different viruses of the potato mosaic-crinkle-streak group is not quite so large as current descriptions would lead one to expect.

Another point of interest lies in the difference in receptivity of the various plant species to the ringspot virus when transmitted by the aphis *Myzus persicae*. It is fairly certain that the insect picks up the virus, as it is able to infect both *Datura* and *Capsicum* when brought from infected *Datura*, but so far all attempts to infect most of the other solanaceous plant species with the ringspot virus by means of *M. persicae* have failed. This phenomenon has been shown already by the writer's studies on the transmission of potato viruses by this aphis (6). Although it was found difficult to infect potato plants with mosaic and crinkle by means of *M. persicae*, yet by the development of a disease in tobacco plants colonised with some of the same series of insects the aphis was proved to be picking up the virus or at all events *a* virus, possibly part of a virus complex. Similarly, when *M. persicae* was colonised upon a potato plant infected with a combination of leaf-roll and mosaic, and then transferred, some to healthy potato and some to healthy tobacco, only leaf-roll developed in the potato, but the development of a disease in the tobacco, which is immune to leaf-roll, showed that the insect was at any rate picking up two viruses (4).

In cross-inoculation studies with several plant viruses the writer has been impressed by the apparent variation in the degree of infectiousness of the same virus when in different plant hosts. The ringspot discussed in the present communication affords an illustration of this; it is much more easily disseminated when in tobacco or *Datura* spp. than when in *S. capsicastrum* for example. Further, certain potato viruses of the mosaic-crinkle group appear to be more easily communicable to other potato varieties and other plant hosts after passage of the tobacco plant than before passage of that plant (6). As an example the case of a virus-carrying Di Vernon potato may be cited; needle inoculation into *Datura stramonium* from this plant consistently failed to produce symptoms, but if the virus were first inoculated into White Burley tobacco, in which it produced a severe ringspot disease, and then transferred to *Datura*, infection of this plant was easily accomplished. It might almost be suggested that passage of certain plant species "attunes" or "educates" a plant virus within narrow limits to attack other plants to which it is normally non-transmissible, a phenomenon somewhat similar to the "bridging host" theory of Marshall Ward in relation to certain rust

fungi. This apparent "adaptation" of a virus by passage of other plants may, however, consist merely of the production of an alteration in virulence, possibly accompanied in some cases by the holding back of one component of a virus complex.

The phenomenon of temporary recovery of tobacco plants affected with the virulent form of this ringspot virus is very well illustrated in the present study. It sometimes happens that the plant dies down except for the central shoot, and this in time produces a new plant devoid of all symptoms and apparently healthy. This plant will grow normally for 4 to 5 weeks, after which the severe symptoms break out anew and the plant collapses again. This may happen two or three times, after which rings and necrosis appear again and the plant remains in a stationary and moribund condition. The same reaction has been shown by the writer to take place in the case of the severer forms of ringspot disease induced by needle inoculation of tobacco with certain potato mosaic viruses (5).

V. SUMMARY.

1. A ringspot virus affecting solanaceous plants is described. This is the first record of the disease in the British Isles.

2. The reactions of the following solanaceous plants to the ringspot virus are described: *S. capsicastrum*. *S. tuberosum* (potato). *S. laciniatum*. *S. nigrum* (black nightshade). *S. nodiflorum*. *Lycopersicum* (tomato). *Nicotiana tabacum* (var. (*a*) White Burley, (*b*) Virginia). *N. glauca*. *Datura stramonium* (jimson weed). *Capsicum* spp.

3. The close parallels in the behaviour of the ringspot to the viruses of the potato mosaic group are emphasised. The virus is able to exist in a variety of symptom expressions according to species of host plant and degree of virulence. "Ringspot" is thought to be an alternative symptom expression for a number of mosaic viruses, the development of the ringspot symptoms probably depending upon the degree of virulence of the virus, the type of host plant and the method of infection of that host plant.

4. A marked difference in receptivity of the various solanaceous plants to the ringspot virus when transmitted by the aphis *M. persicae* is shown to exist. The virus was transmitted by means of this aphis to *D. stramonium* and *Capsicum* and not to the other species.

5. The phenomenon of temporary recovery of tobacco plants affected with the ringspot in its virulent form is well illustrated. Plants die down and then re-grow for a period, temporarily symptomless, only to succumb once more to a severe attack. This may happen two or three times before the plant finally remains in a necrotic and moribund condition.

6. In no case was the virus transmitted through the seed of affected plants.

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EXPLANATION OF PLATES I—V.

PLATE I.

Figs. 1, 2. The expression of the ringspot virus on *S. capsicastrum*. Note the very striking pattern of rings and diamonds. Fig. 2 shows some general necrosis of the leaf tissue between the rings. Length of leaves, 8–9 cm.

PLATE II.

Fig. 1. Leaf of White Burley tobacco affected with the ringspot virus. In this particular case the symptoms are confined to one side of the leaf. Length of leaf, 15 cm.

Fig. 2. Rings forming near the inoculation scratches on a leaf of White Burley tobacco. Note the tendency of the rings to form round the veins. Length of leaf, 8 cm.

PLATE III.

Fig. 1. Ringspot in White Burley tobacco produced by needle inoculation from potato Di Vernon which was "carrying" a virus. Note the similarity in symptoms to those shown in Fig. 2. Length of leaf, 10 cm.

Fig. 2. White Burley tobacco affected with the ringspot virus from *S. capsicastrum*. Compare with the symptoms shown in Fig. 1 due to a potato virus on tobacco. Slightly lower magnification than in preceding figure.

Fig. 3. Leaf of older plant of White Burley tobacco, showing the general necrosis of the veins caused by the ringspot virus. The "ring" element is still present. Note that the young leaves are so far symptomless. Length of leaf, 16 cm.

Fig. 4. Leaf from mature White Burley tobacco affected with the ringspot virus in a virulent form. Note the severe necrosis and "corkscrewing" of the leaf. Length of leaf, 12 cm.

PLATE IV.

Fig. 1. Two White Burley tobacco plants affected with the ringspot virus in its most virulent and "streak"-like form. The continual killing out of the growing points is shown. Height of plants, 2 and 1½ ft. respectively.

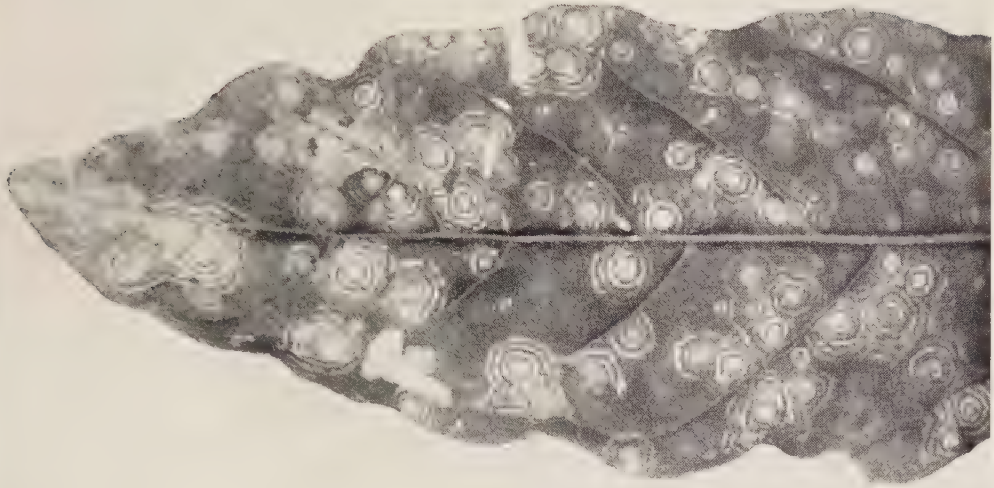

Fig. 1.

Fig. 2.

SMITH.—STUDIES ON POTATO VIRUS DISEASES (pp. 1–15).

Fig. 1.

Fig. 2.

SMITH.—STUDIES ON POTATO VIRUS DISEASES (pp. 1–15).

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

SMITH.—Studies on Potato Virus Diseases (pp. 1–15).

Fig. 1.

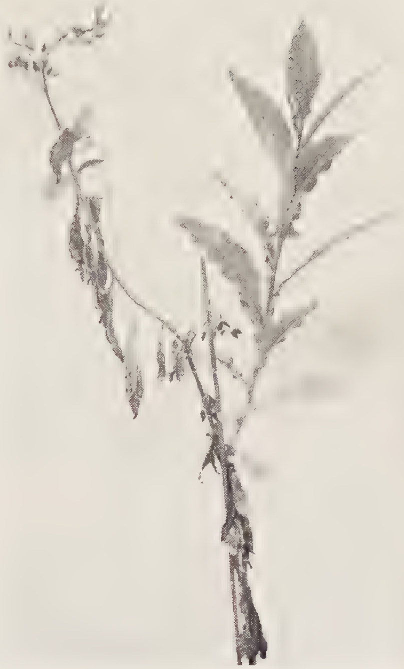

Fig. 2.

Fig. 3.

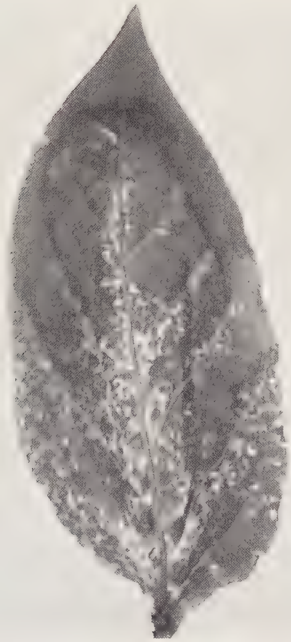

Fig. 4.

SMITH.—STUDIES ON POTATO VIRUS DISEASES (pp. 1–15).

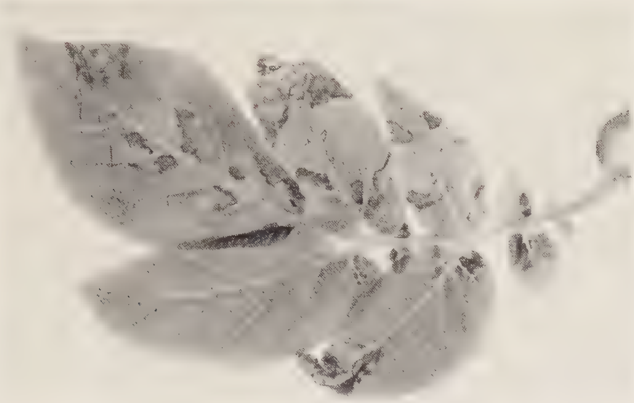

Fig. 3.

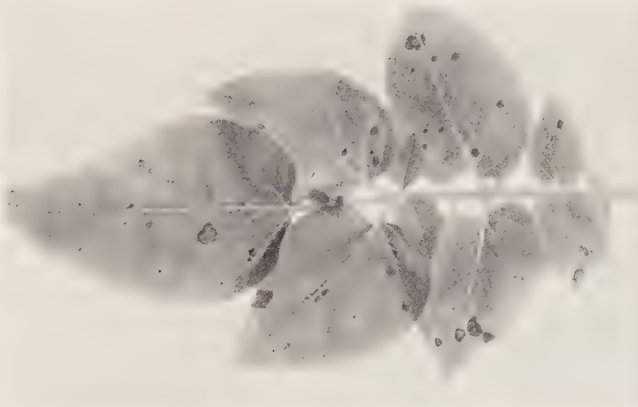

Fig. 2.

Fig. 1.

SMITH.—Studies on Potato Virus Diseases (pp. 1–15).

Fig. 2. Tobacco, White Burley, showing the production of a symptomless and apparently virus-free shoot from the badly diseased parent stem. Height of plant, 4 ft.

Fig. 3. Leaf of *D. stramonium* affected with the ringspot virus which is here shown as a mosaic. Note the curious "snakeskin" effect. Length of leaf, 10 cm.

Fig. 4. Leaf of *Capsicum* affected with the ringspot virus. In this plant the symptoms may be either in ring form or a "speckle" mosaic as shown. Length of leaf, 10 cm.

PLATE V.

Fig. 1. The ringspot virus as it appears on *S. laciniatum*, numbers of large *single* rings of a purple colour develop on the leaves. The growing point becomes twisted and distorted. Length of leaf, 15 cm.

Fig. 2. The ringspot virus on potato Arran Victory, early stage of infection; at this stage fairly clear rings may develop.

Fig. 3. The ringspot virus on potato, later stage; here a general necrosis of the leaves reminiscent of potato streak has set in.

(Photographs by C. W. Williamson.)

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APHIDES AS VECTORS OF "BREAKING" IN TULIPS

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(With Plate VI.)

Introduction.

In 1928 Miss D. M. Cayley (1) brought evidence to show that the phenomenon known as "breaking[1]" in tulips was due to the action of a virus, and demonstrated that "breaking" was transmissible both by grafting and also by inserting a plug of tissue from a "broken" bulb into one known to be of pure stock. By analogy with other virus diseases it seemed likely that the natural vector for "breaking" in tulips would prove to be an insect, probably an aphis, and therefore in 1928–9 experiments were carried out with four species known to feed upon tulips. They were *Anuraphis tulipae* B. de Fonse, *Rhopalosiphoninus tulipaella* Theo., *Macrosiphum gei* Koch, and *Myzus persicae* Sulz (2). In these experiments the two former species gave negative results, *M. gei* induced "breaks" amounting to 5·6 per cent., but in the case of *M. persicae* the proportion of "breaks" was much higher, amounting to no less than 25 per cent. It appeared desirable that the positive results with the two latter species should be confirmed by experiments carried out on a much larger scale, and in 1929–30 therefore only *Myzus persicae* and *Macrosiphum gei* were used, with a considerably larger quantity of material.

General description of the experiments.

The bulbs used in these experiments were obtained from the following sources:

Stock bulbs as sources of the virus:

30 Sulphur "broken," from the garden of the John Innes Horticultural Institution.

13 Bartigon "breaks" induced by *Myzus persicae* in 1928–9.

Bulbs used for the experiments:

2000 Bartigon "breeders" from Cambridgeshire.

[1] "Breaking" is the term which denotes the occurrence of flecks and stripes in the flowers of tulips, instead of the pure "self" colour of the "breeder" tulip. These stripes normally represent the segregation of the colour in the epidermal layer, leaving elsewhere the white or yellow colour of the mesophyll to show. In the "red breaks" here described the segregation is only into lighter and darker shades of the breeder colour.

It is impracticable to ensure an experimental stock of tulip bulbs that shall be absolutely free from infected bulbs. In the best Lincolnshire and Dutch bulb gardens there is a very small proportion of "breaks," which are carefully rogued out each year. Aphis is not, however, entirely absent and some transmission of the virus may therefore occur. Since it would take some years and very elaborate precautions to build up a stock that had been protected from all possibilities of infection, it is more practicable to work with a good commercial stock in large enough numbers to swamp the experimental error due to a few infected bulbs in the stock. As will be seen this error is less than 1 per cent. Some of the bulbs had to be discarded because they were infected with the eel-worm, *Tylenchus dipsaci*.

All the bulbs were planted in separate 6 in. pots and plunged under ashes in the open, the stock bulbs on September 17th, 1929, and the experimental bulbs on October 1st. The stock bulbs were brought into the intermediate house (temperature 60–65° F.) on December 9th, and infected with *Myzus persicae* and *Macrosiphum gei*, each plant being covered with a hurricane lamp glass with a muslin top. On January 1st, 1930, the experimental plants were brought into a green-house rendered insect proof, and maintained at a temperature of 50° F. During the week following each pot was covered with a separate hurricane lamp glass with muslin top, and the temperature gradually raised to 60–65° F. The first infection with aphides from the stock plants took place on January 8th, 1930, and in the following 6 days aphides were transferred to the experimental plants until all had been infested. In infesting the plants with aphides, never less than ten and, as a general rule, a considerably greater number, were placed on each plant. After the aphides had been allowed to live and breed upon the plants for a fortnight, each group of pots was removed to a fumigating chamber where the lamp glasses were removed and the plants fumigated with nicotine. Each group remained in the fumigating chamber over night, and was brought back without the glass covers next morning. When all the plants had been fumigated, with the exception of the controls which had also been covered with lamp glasses throughout the period of infestation in the experiments, these glasses were also removed and the whole house was fumigated. This practice was continued twice a week until it was no longer necessary.

The colonies of *Myzus persicae* and *Macrosiphum gei* were reared throughout the summer on radish and lettuce respectively, and were the direct descendants of the stocks used in 1928–9.

Methods of recording intensity of "break."

In these experiments and in those of 1929 the types of "break" dealt with were of two kinds, namely "white break," in which streaks and stripes of white occur, and "red break," in which deeper red streaks are observed on the red ground colour, these latter were the predominant feature of these experiments. The intensity of "breaking" was recorded in six degrees, – – –, – –, –, +, + +, + + +, but for the purpose of this paper all "– breaks" will be recorded together under the heading "slight breaks," and all "+ breaks" under the heading "breaks." In all cases discards before scoring, "blinds" and "rogues" were excluded from the percentages.

Detailed description of the experiments.

Control. 500 bulbs of the tulip variety Bartigon were set aside as control for all the experiments. They were kept in a separate compartment, which had been specially built so as to be as far as possible aphis proof, in the same house as the other plants. Lamp glasses over each plant were used as an additional safeguard during the infestation period. No aphides appeared on the control plants at any time during the course of the experiment. When the plants came into flower, one showed a definite "white break," and three flowers exhibited red flecks not quite like the usual forms of "red break," but which may nevertheless prove to be "breaks" when the bulbs flower next year.

Summary of results.

Normal	468
Blinds	22
Rogues	2
Discards	4
"Breaks":	
Definite	1
Possible	3
Total	500
Percentage "breaks":	
Definite	0·21
Possible	0·85

The following experiments were undertaken with *Myzus persicae*, the infections were all carried to tulips of the variety Bartigon.

Experiment A. From Sulphur "broken."

Experiment B. From thirteen bulbs of Bartigon in which "breaking" had been induced by *Myzus persicae* the previous season.

Experiment E. From pure "self" Bartigon.

Experiment A.

300 bulbs were used in this experiment. On January 8th, 200 plants were infested with aphides from "broken" Sulphur, the remaining 100

being treated the following day. The aphides were allowed to colonise these tulips until the evening of January 21st and 22nd respectively, when each group was fumigated. The first flower appeared on February 1st. A phenomenon (first noticed in Experiment *B*) must be mentioned as it subsequently occurred in most of the other groups. A flower, fully opened, when first examined appeared in all respects normal, but 4 days later showed all the signs of intense "red break." The effect of the virus became evident in this short period although the flower was already mature. In very many cases in Experiment *A* the "breaking" became more intense after its first appearance. For example, a flower recorded as a "slight red break –" on February 18th was scored as a "red break +" on the 25th; another recorded as a "very slight red break – –" on February 18th had become "red break + +" on March 6th; a third scored as "red break +" on February 20th had become "red break + + +" on March 6th.

Summary of results.

Normal		89
Blinds		35
Rogues (all "broken") ...		3
"Slight breaks" ...	50	
"Breaks"	123	
Total "breaks" ...	——	173
Total		300
Percentage of "breaks" ...		66·03

Experiment B.

In the season 1928–9 *Myzus persicae* induced "breaking" in thirteen Bartigon tulips. Five were "red breaks," four were "red breaks" with a little white, four were both "red" and "white breaks." These plants were grown on this year and used for infection purposes in this experiment. Two hundred bulbs were used in this group. The plants were infested with aphides on January 9th and 10th, 1930, and fumigated on the evening of January 22nd and 23rd respectively. The first plant to flower was fully open on February 1st and was normal. On February 5th it exhibited heavy "red break + +"; the final results were as follows:

Summary of results.

Normal		166
Blinds		16
Rogues ("broken + +") ...		1
"Splash"*, etc.		3
"Slight breaks" ...	6	
"Breaks"	8	
Total "breaks" ...	—	14
Total		200
Percentage of "breaks" ...		7·65

* "Splash" is the term used to denote flowers showing an isolated sector of darker red upon the red ground. The phenomenon is probably unconnected with "breaking."

Experiment E.

100 plants were set aside for this experiment, which was in the nature of a control, since its object was to prove that the "breaking" phenomena were not carried by aphides which had fed upon "unbroken" material. To test this, some of the over-wintered aphides were fed upon thirty bulbs taken from the Bartigon stock, and presumably uninfected. Unfortunately, of these thirty, three showed definite "red breaks" when they flowered, thus rendering the whole experiment inconclusive. Of the 100, eighty-three flowered, two were rogues ("broken"), four showed "red break," the rest were "unbroken."

The following experiments were undertaken with *Macrosiphum gei*, and all infections were carried to tulips of the variety Bartigon:

Experiment X. From Sulphur "broken."

Experiment Y. From pure "self" Bartigon.

Experiment X.

302 plants were used in this experiment. Aphides were transferred from plants of "broken" Sulphur on January 13th, 1930, and fumigation took place on the evening of January 26th.

Summary of results.

Normal		142
Blinds		39
Rogues		5
Discarded before being scored ...		12
"Slight breaks" ...	42	
"Breaks"	62	
Total "breaks" ...	—	104
Total		302
Percentage of "breaks" ...		41·63

The percentage of "breaks" in this experiment was less than that in the parallel experiment with *Myzus persicae*, thus bearing out the observations made last season that *M. gei* is a vector of "break" in a lesser degree than *M. persicae*, although nevertheless an important vector. The association suggested in 1929 of "white break" with *M. gei* and "red break" with *M. persicae* has not been borne out this season, as both species of aphides induced these two types of "break" this year.

Experiment Y.

100 plants were used in this group and pure "self" Bartigon was the stock variety for infection. On flowering none of this stock proved to be "broken." The plants were infected on January 14th, 1930, and fumigated on the evening of January 27th. One flower showed very slight signs of

"break," and one flower was a "sport," somewhat resembling the variety Sundew.

Summary of results.

Normal	86
Blinds	12
Sport	1
"Slight break"	1
Total	100
Percentage of "breaks" ...	1·14

The previous experiments were carried out under glass, which ensured that infection and flowering took place before there was any movement of aphides in the open. The following experiment was made in the open, with a bed of Clara Butt which had been planted in the autumn of 1929 from a stock obtained from Lincolnshire. The bed contained 1330 plants and, in April 1930, when the plants had made a few inches growth, but before any aphides had appeared in the open, a muslin-covered cage divided into three equal compartments was erected over the whole bed. The cage was not absolutely aphis proof; at a later stage a good many *M. gei* were observed on plants in the control compartment. They were duly kept down and, as far as may be judged from this year's flowering, carried no infection.

Mass infections were made in the two end compartments, the central compartment being kept as control. The aphides *Myzus persicae* and *Macrosiphum gei* were again used, and were distributed as far as possible evenly over the plants in their respective compartments. By this method, however, it cannot be said that each plant had a definite minimum number of aphides, and it is possible that some plants did not receive any. The stock plants for infection of the aphides were again "broken" Sulphur.

The control experiment.

The controls were all normal tulips of the variety Clara Butt, with the exception of one "white break." This "break" was the last to flower, and was at no time infested by aphides.

Summary of results.

Normal	430
Blinds	15
"Breaks"	1
Total	446
Percentage of "breaks" ...	0·23

The experiment with Myzus persicae.

There were 441 plants in this compartment. The aphides were allowed to feed on the foliage of "broken" Sulphur tulips, and were then dis-

tributed as evenly as possible over the whole compartment. Infestation took place on April 9th, 1930 when the tulips were in bud and were already showing signs of turning pink. When the flowers were fully open many were found to be "broken." They were all of the same type of "red break" in varying degrees of intensity, some only showing slight red streaks on one or more petals, others, the most intense "breaks + + +," became pure red tulips very similar in colour to Bartigon. The intensity of the infection increased as the flowers matured, which appears to be the general tendency in this type of infection. The results were as follows:

Summary of results.

Normal	270
Blinds	6
Rogue ("broken")	1
"Breaks"	164
Total	441
Percentage of "breaks" ...	37·79

The experiment with Macrosiphum gei.

This experiment was in all respects similar to that with *Myzus persicae*, although the "breaks" in this case were noticeably less intense. The type of "red break" was the same in each case.

Summary of results.

Normal	337
Blinds	3
"Breaks"	103
Total	443
Percentage of "breaks" ...	23·40

Later development of 1928–9 *bulbs.*

The bulbs of the 1928–9 experiments were harvested and kept in bags in the general bulb store. After some time many of them were found to be infested with *Anuraphis tulipae* and, to a lesser degree, with *Myzus persicae*. The results, therefore, are subject to possible error from infection at this stage and are given with reserve, but they indicate progress in "breaking." The plants were grown in the open, and only three or four flowers on the outside row, which were normal, were attacked by *Macrosiphum gei* before scoring took place.

Summary of results.

Normal	93
Blinds	16
"Slight red breaks"	3
Total	112
Percentage of "breaks" ...	3·13

Bulbs infected by Myzus persicae *in* 1928–9.

All the plants which had shown "broken" flowers last year were used for infection purposes in Experiment *B*, and as a result of aphis damage only four of the thirteen flowered. Of these, two "red breaks" of 1928–9 showed this year flowers with a heavy "white break," an indication that "red break" may be an intermediate stage of virus infection, the full "white break" taking longer to mature. Of the plants infected in 1928–9 but which flowered "unbroken," three were blind, twenty-six again gave "unbroken" flowers, but ten were "broken," eight being "white breaks" and two very slight "white breaks."

Of the infected plants which were blind in 1929, twenty-two flowered this year, eighteen of them being "white breaks."

To summarise, whereas the percentage of "breaking" in the year of infection had been 25 per cent. only—all "red breaks," in the following year it had progressed to 48·28 per cent., 45 per cent. being "white breaks." Too much weight must not be attached to this result, because of the possibility of a second infection in store.

Bulbs infected by Macrosiphum gei. All the "white breaks" which appeared last season again flowered this year, and showed slightly heavier "breaking." One plant noted last year as "splash" gave "white break + + +" this season, but the other two "splashes" returned to normal. The very slight dark marked flower of last year gave a "red break + + +," having thus increased the degree of "breaking," although not showing "white break." Of last season's normal bulbs, thirty-seven gave normal flowers, two blinds, and one "white-and-red break +." The proportion of "breaks" among the plants which were blind last season was much higher; sixteen normals, eleven blinds and six "breaks" all exhibiting "white breaking" to a very marked degree.

Bulbs infected by Anuraphis tulipae. Sixty-three bulbs which had been infested last year with this species after it had fed upon "broken" Darwin tulips, again flowered this year, and two only showed very slight "red break."

Bulbs infected by Rhopalosiphoninus tulipaella. Sixty-one bulbs infested with this aphis in 1928–9 again flowered in 1930, three only showing "red break."

Parrot tulips.

Experiments were started to ascertain if the abnormal appearances presented by Parrot tulips were also the products of some kind of virus infection. Parrot tulips have been known since the early part of the

seventeenth century, and are characterised by curled and laciniated petals in which are inclusions of green tissue. The origin of a few varieties in general cultivation, which are bizarres, *i.e.* yellow ground tulips with red or brown colour superimposed, is unknown. There is a common statement that seeds give rise to ordinary tulips, but no positive evidence is available, and as both ovary and pollen are commonly imperfect this popular opinion may well possess no basis. Of recent years a few new varieties of parrot tulips have occurred as sports of known varieties. For example the parrot tulip Fantasy is a sport of Clara Butt, and in its colouring and other features clearly belongs to that variety. Both "broken" and "self" parrot tulips are known. It seemed possible that the "parroting" characteristics might be the result of a virus or a combination of viruses, especially as slight laciniation of the perianth segments has occasionally been observed to accompany "breaking." It is, however, unlikely that "parroting" is due to any widespread virus, since its occurrence has been so rare among the enormous quantities of tulips grown. Miss D. M. Cayley has so far failed to transmit "parroting" by grafting a portion of a living parrot bulb to a normal bulb, in the manner in which she demonstrated the transmission of breaking.

Aphides for infestation were fed as follows:

Experiment C. On the parrot tulip Markgraaf van Baden.

Experiment D. On both "broken" Sulphur and Markgraaf van Baden.

Experiment C.

There were 150 Bartigon tulips in this experiment and the plants used for infection were the parrot tulips Markgraaf van Baden. The aphides were transferred to this group on January 10th–11th, 1930 and the plants were fumigated on the evening of January 23rd–24th. When the flowers appeared four exhibited slight red flecking, probably "red break," but as the controls also contained plants which were very slightly flecked, too much importance should not be attached to this result. There was no sign of "parroting" this year, but as the plants flowered at an interval of only 3 to 6 weeks from the day of infection it is possible that the change of form such as "parroting" necessitates might not have had the requisite time for action. The bulbs will be planted next year to see if any change takes place. It should be noticed that there is no evidence at present that "parroting" is due to a transmissible virus, or that the two aphides used in these experiments are the natural vectors in this instance,

should "parroting" subsequently be proved to be due to such a cause.

Summary of results.

Normal	136
Blinds	8
Rogue (normal)	1
"Splash"	1
"Slight breaks"	4
Total	150
Percentage of "parroting" ...	0
Percentage of "breaks" ...	2·86

Experiment D.

100 plants were used in this group. The stock plants for infection were both "broken" Sulphur and Markgraaf van Baden, not less than ten aphides from each source being placed on the experimental Bartigon tulips. Infestation took place on January 11th, 1930 and the plants were fumigated on the evening of January 24th. The object of this experiment was to ascertain, supposing "parroting" should prove to be a transmissible virus, whether the mixture of the two infections, "break" and "parrot," would produce a different result from the infections when transmitted separately. As far as "parroting" was concerned there was only one flower which showed any signs of attenuation or serration and that in only one of the petals, and it is very doubtful if even that will persist next season. In this flower the normal six petals were present and one of them was streaked with green, a character of parrot tulips; but this phenomenon is also found in "self" tulips where a bract has become elongated to take up a position in close association with the petals, and where part of the green colour is replaced by red. In most cases where this happens, these precocious bracts are supernumerary to the normal six petals. In this flower, although there were certain preliminary characters which might be associated with "parroting," it can in no way be claimed that this state had been induced as a result of aphid transmission. One flower of this group exhibited the "white break" on the outside of the petals and the "red break" inside. Many of the flowers in this experiment were stunted and slightly different in shape and colour to the other groups, differences which are very difficult to describe. The results again are difficult to explain, as the "breaking" in this group is nearly 19 per cent. higher than transmission from "broken" Sulphur and more than 82 per cent. higher than transmission from Markgraaf van Baden. It may be suggested that this was due to a greater number of aphides being placed on each plant, but in many of the other groups an equal number of aphides, if not more, were

transferred to some plants without any marked difference in infection being perceptible. It is possible that there is a strain of "breaking" in Markgraaf van Baden which is not easily transmissible by itself, but which when mixed with the infection from "broken" Sulphur develops to an abnormal degree. Or again, the possibility of two viruses must not be lost sight of; further experiments on this aspect of the case will be carried out in 1931 to try to elucidate the matter.

Summary of results.

Normal		14
Possible "parrot"		1
Blinds		6
"Slight breaks"	24	
"Breaks"	55	
Total "breaks"	—	79
Total		100
Percentage of possible "parrot"		1·08
Percentage of "breaks"		84·95

Bulbs infected by Macrosiphum gei. *Experiment Z.*

There were 150 plants in this experiment and the stock plants were the parrot tulip Markgraaf van Baden. Infestation took place on January 14th, 1930 and fumigation on the evening of January 27th. There were no signs of "parroting," although one flower was a very slight "red break."

Summary of results.

Normal	134
Blinds	14
Rogues	1
"Slight break"	1
Total	150
Percentage of "parrot"	0·00
Percentage of "break"	0·74

Experiment A.V.M. From mosaic infected Arran Victory potatoes.

The potatoes were planted on October 20th, 1929, and when the haulms appeared they were infested with *Myzus persicae*. In other respects this experiment was in every way similar to the others. Arran Victory mosaic potatoes received from Dr R. N. Salaman were used for infection purposes. By forcing the tubers too quickly the haulms became very weak and the aphides did not colonise well on them. As a result only thirty-two Bartigon tulips could be infested on January 10th, 1930. They were fumigated a fortnight later on the evening of January 23rd. The results were negative.

Summary of results.

Normal	30
Blinds	2
Total	32
Percentage of "breaks"	0·00

Experiments continued from 1928–9.

In 1929, about the end of April, Arran Victory mosaic tubers were received from Dr R. N. Salaman of Cambridge, and aphides of the species *Myzus persicae* were fed upon the growing shoots and afterwards transferred to tulips of the variety Bartigon, which were almost in full flower. Owing to the cage becoming torn the controls were also heavily infected, but no results were obtained during the year. In the present season 1930 neither the controls nor the treated plants showed any signs of abnormality, and it would appear from this and from the experiment A.V.M. that mosaic disease of the potato is not transmissible to tulips by this aphis. Among the treated plants were thirty-one normals, three blinds and no "breaks," and in the control twenty-nine normals, no blinds and no "breaks." Percentage of breaks 0·00.

Discussion.

This season's experiments afford strong confirmation of the results obtained previously—that the virus of "breaking" is carried from "broken" to normal tulips by the two species of aphis, *Myzus persicae* and *Macrosiphum gei*, more freely by the former than by the latter. In the 1930 trials a higher percentage of infection was obtained than in 1929. Under glass (Experiment *A*), with *Myzus persicae* carrying infection from "broken" Sulphur, 66 per cent. of "breaks" were obtained as against 25 per cent. in 1929. In the open 38 per cent. of "breaks" were obtained.

With *Macrosiphum gei* the percentages were 41·6 and 23·4 respectively against 5·6 in 1929.

Possibly these results may have been influenced by the source of infection. In the 1929 experiments the aphides used for infection had been fed on a mixed lot of "broken" tulips, in 1930 they were fed only upon "broken" Sulphur, a variety known to be specially susceptible to "breaking." In Experiment *B* the aphides (*Myzus persicae*) had been fed on "broken" Bartigon, and this infection was only followed by 7·65 per cent. of "breaks." Further experiments are required to test this question of whether the virus from one variety of tulip is more virulent than that from another. One other experiment seems to suggest variations in virulence. The highest percentage of "breaking," 85 per cent., was obtained when tulips were infected both with aphides that had fed upon "broken" Sulphur and upon the parrot Markgraaf van Baden, though the latter alone induced but a small proportion of "breaking," almost covered by the probable error.

There was in this season's experiments a distinct suggestion of a time factor in the extent and the nature of "breaking." In the 1929 trials the infections with *Myzus persicae* induced thirteen "breaks," four of which were typical red and white, five were "red breaks," while four were "red breaks" showing a little white. In 1930 among the much larger number of "breaks" a few showed white which did not change, but the intensity of "red breaking" increased as the flowers aged, and in many cases flowers which opened with little sign of "breaking" were scored later as distinct "breaks." The time interval between infection and flowering was longer in 1929, namely, 6 weeks. In 1930 the infection period was from January 8th–27th. Some flowers began to open on February 1st; scoring began on that date and was completed on March 20th, though the majority were scored in February or early March. The average interval between infection and flowering was thus little more than a month. Similarly, in the open air experiment, infection only began on April 9th, 1930, and the tulips were in full flower at the end of the month. The progression in "breaking" is also to be noted in the bulbs infected and showing "breaking" in 1929; in most cases "red breaks" of 1929 became "white breaks" in 1930. Again some of the plants infected in 1929 but which showed no "breaking" that year became "white breaks" in 1930.

In nature *Myzus persicae* only reaches the tulips comparatively late in the season, *i.e.* when in full flower. The infection does not show that season, but, having had time to develop itself during the period of resting and early growth, manifests itself in the following season as a full "white break." Further observations are necessary before the sequence from slight to full "red break" and thence to "white break" can be regarded as established, as also on the nature of the "breaking" without any white exhibited by certain deep red or dark purple varieties.

Macrosiphum gei appears in the field somewhat earlier than *Myzus persicae*, but rarely before the tulips are in flower.

These partial conclusions as to the time of infection should be read as applying only to the late May flowering tulips. The early varieties, which usually flower in April, at least a fortnight ahead of varieties like Bartigon, are usually free from "breaking," but are not immune, as both observation and experiment have shown. Possibly in a normal season, by the time that either of the aphis species appears, they may have reached a stage of maturity that does not allow an infection to be carried to the new bulbs. On this point, the time of infection in the field, more experiments have been designed.

Fig. 1.

Fig. 2.

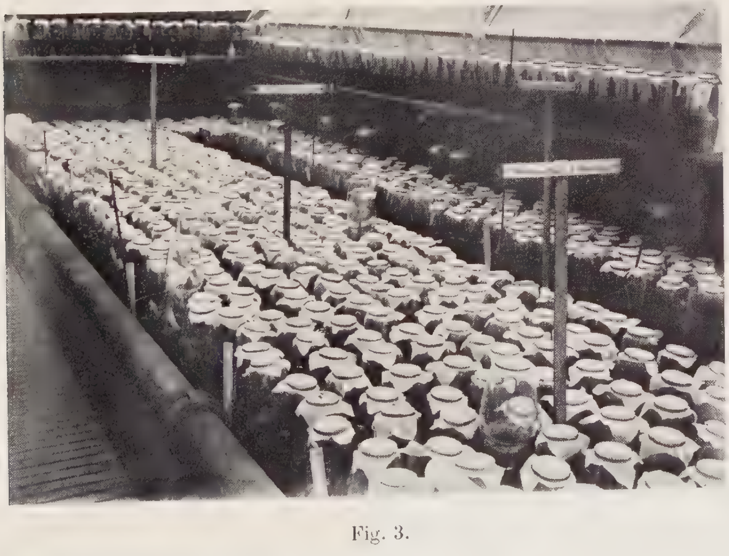

Fig. 3.

HUGHES.—Aphides as Vectors of "Breaking" in Tulips (pp. 16–29).

Summary.

1. *Myzus persicae* Sulz is a vector of "breaking" in tulips.

2. *Macrosiphum gei* Koch is also a vector of "breaking" in a lesser degree.

3. "Red break" and "white break" are transmitted equally by the two species.

4. It is suggested that "red break" is an earlier stage in the development of the virus and that "white break" is the final stage.

5. There is at present no indication that "parrot" is transmissible by these two aphid species, though no conclusion can be reached in one season.

6. There is some indication that the virus can be transmitted from some varieties of tulips more readily than from others.

7. Mass infection in the open gave a lower percentage of "break" than individual infection under glasshouse conditions.

8. No conclusive evidence has been obtained to show that *Anuraphis tulipae* B. de Fonse and *Rhopalosiphoninus tulipaella* Theo. convey "breaking," but in any case they cannot be considered as important vectors.

Acknowledgments.

Thanks are again due to Sir Daniel Hall for his help and advice; to Mr J. C. F. Fryer, Dr G. H. Pethybridge, Dr J. Henderson Smith, Dr R. N. Salaman, Prof. J. W. Munro, Mr F. Laing, and many others who have all been most helpful in supplying material or assisting in the interpretation of the results.

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EXPLANATION OF PLATE VI

Fig. 1. Progress of "breaking" induced by *Myzus persicae* in 1929.

Fig. 2. Red "break" in Clara Butt.

Fig. 3. View of part of the house during the infestation period.

(*Received July 3rd*, 1930.)

STUDIES IN BACTERIOSIS

XVIII. A BACTERIAL CANKER OF APPLE TREES

By MARGARET S. LACEY and WALTER J. DOWSON.

(From the Bacteriological Laboratory of the Imperial College, South Kensington, and the Royal Horticultural Society's Gardens, Wisley, Surrey.)

(With Plate VII.)

The disease about to be described occurred among seedling apple trees at Wisley, and has occasioned the replanting of a considerable number of these during the past few years. The disease was first noticed by Mr A. N. Rawes, the pomologist at the Wisley Gardens, and shown to one of us (W. J. D.) early in 1923. Several new varieties of apple trees had been planted and some of these exhibited the disease to quite a marked degree, the variety Premier being the most susceptible to attack.

The disease was characterised by cracks in the bark, horse-shoe shaped or circular in outline, and varying in size from $\frac{1}{8}$ to 1 in. across (Plate VII, fig. 1 *A*). They were arranged either in groups or singly, the smaller ones being the more numerous. Scattered among these latter was a number of small raised swellings or blisters, $\frac{1}{8}$ in. in diameter (Plate VII, fig. 1 *B*). In October 1923 the trees were pruned for the first time, and it was then found that on about half of this particular variety (Premier) of which there were forty trees, many of the buds, including terminals, had been killed, and that surrounding the leaf scars below such buds were brown depressed areas outlined by a crack in the bark (Plate VII, fig. 2 *D*). While searching for healthy buds to which to cut back, markings similar to the small horse-shoe shaped depressions already noted on the main stem, were found on the internodes of the branches of one tree. On the same shoots there were elongated depressed areas ranging from $\frac{1}{4}$ to 1 in. in length, separated from the rest of the bark by slight but distinct cracks. From some of these lesions the epidermis was peeling off in brown flakes (Plate VII, fig. 2 *A*), and the whole trouble was ascribed at the time to a combined attack of canker and scab. At a later date, however, when such lesions were examined microscopically, no traces of fungal hyphae likely to account for the pockets of dead cells

within the cortex could be found. On the other hand the appearance of the necrotic areas strongly suggested a bacterial invasion. The leaves dropped off the affected trees earlier than did those of surrounding trees of the same variety, and, although all the affected shoots so far as could be seen had been cut out, the shape of the young trees being quite spoilt thereby, yet by November 1923 similar markings had appeared on the stumps of those shoots. The shoots were again shortened, but apparently without removing the course of infection, for the same kind of lesions appeared in the following season (1924) on buds, leaf scars, and internodes. No girdling of the main stem or branches has occurred, and the extension of the diseased tissues has been in a vertical rather than in a horizontal direction[1]. The actual dying back has been but slight, involving only the terminal inch or so, though many dead buds and patches from time to time have been found along the shoots.

EXAMINATION OF DISEASED TISSUE.

Upon cutting into any one of the lesions described above, including the "blisters," brown specks and streaks were found embedded in the cortical tissue. In some cases brown streaks ran vertically in the cortical layers for a considerable distance from the original surface lesion, occasionally breaking through to the surface in minute blisters. In other cases, the only external symptom was the appearance of light brown patches on the epidermis, but on cutting beneath these the brown necrotic lesions were found in the cortex. In natural infections the disease was mainly confined to the bark, and macroscopically the wood appeared sound, although on microscopical examination the vessels in the neighbourhood of a cortical lesion were sometimes found to be plugged with a thick gummy substance.

On the other hand when inoculations of the causal organism (described below) were made extending into the wood, infection progressed vertically, here as well as in the cortex in both directions, and extensive brown streaks became visible to the naked eye. Microscopical examination showed that many of the wood-parenchyma cells were destroyed and, in addition, some of the vessels were filled with gummy substance and others with bacteria.

ISOLATION OF A CAUSAL ORGANISM AND INOCULATION EXPERIMENTS.

Numerous isolation plates have been made at various times from the different types of lesions, and in most cases (the failures being from old

[1] This is in contrast with the characteristics of a disease occurring in South Africa and described by C. J. Hopkins (see p. 34).

or dried up lesions) practically pure cultures of bacterial colonies of a single type were obtained. Detailed cultural examinations were made of several of the strains, and they were found to agree except for minor variations. A brief description of the organism is as follows: a short rod, motile by means of one to five bi-polar flagella, markedly aërobic, no spores, Gram-negative, non-acid fast. Colonies on bouillon-agar plates round, raised, entire margin, semi-opaque, shining, white to greyish white, viscous. With some strains a slight green fluorescence is developed in the medium. Gelatine liquefied; no reduction of nitrate; milk becomes alkaline, no clotting, but clearing occurs in zones from above downwards, leaving a clear, straw-coloured fluid. Diastatic action very feeble, acid produced from glucose and feebly from saccharose, none from lactose, maltose or mannite. Indol, some strains negative, others gave a feeble positive reaction. Optimum temperature for growth 25°–29° C., thermal death point 47°–49° C. (10 minutes in standard bouillon), no growth at 37° C.

Numerous prick inoculation experiments were made on apple stems with various cultures of this organism, both at Wisley and at the Chelsea Physic Gardens. Positive results were obtained in most cases, typical small lesions being produced, and the organism was easily re-isolated from necrotic tissue some distance from the point of inoculation. Inoculations were also made on apple twigs placed in flasks of water, the apical bud being cut off with a sharp knife, and cultures of the organism smeared over the cut surface in the manner described by Erwin Smith (7) for inoculation of *B. amylovorus* on pear twigs.

Results.

Some were negative, but in others the top of the twig showed, a fortnight after inoculation, blistering and cracking of the bark for 2–4 cm. below the cut surface, and later, the bark peeled off, exposing brown necrotic tissue below. The appearance was very similar to some of the natural infections developed after pruning. In all cases the controls were completely negative. In a third series of experiments the strains were inoculated on apples in various stages of development from young to practically ripe fruit, but every attempt gave a completely negative result, and inoculation of apple flowers also failed to produce any infection. Lastly, young green apples and pears were inoculated and kept in moist chambers in the laboratory. No rotting occurred.

IDENTIFICATION OF THE CAUSAL ORGANISM AND COMPARISON OF THE WISLEY DISEASE WITH PREVIOUSLY DESCRIBED BACTERIAL DISEASES OF APPLE TREES.

The cultural characters of the Wisley strains appeared to be very similar to *B. amylovorus*, and the authors' attention was drawn to a comparison of the symptoms of disease with those caused by this destructive parasite in North America and New Zealand. The lesions on the internodes described above as elongated depressions outlined by cracks (Plate VII, fig. 3) are very similar to some of the effects of pear blight depicted in numerous American publications. (Compare, for instance, Plate VII, figs. 2, 3, of the present paper with Fig. 35, p. 128, of Duggar's *Fungous Diseases of Plants*, 1911, and with Fig. 115, p. 325, in Bull. 329 of Cornell Univ. (*The Fire Blight Disease of Nursery Stock*, by V. B. Stewart, 1913), or with that on p. 10 of Circular 172 of the Univ. of Illinois (*The Blight of Apples, Pears and Quince*, by B. S. Pickett), or with Fig. 17, p. 43, of Circular 241 of the Univ. of Illinois (*Diseases of Illinois Fruits*, by H. W. Anderson, 1920). These kinds of lesions are, however, the only ones common to the two diseases. The present disease differs from true pear blight in that (1) no killing of the leaves has ever been noticed, (2) no exudation of a bacterial suspension has been observed, and (3) the die-back produced has been but slight. In considering the question of any possible relation to the present disease with fire blight, the differing climatic conditions of Britain and that part of the United States in which fire blight occurs, should be taken into account. Thus at the time when apple trees are in flower the temperature of the United States is considerably higher than that of Britain. Furthermore, the optimum temperature of the fire blight organism lies between 77° and 86° F., and it is interesting to note that the average of the maximum temperature readings for July in that part of Britain where our trees were grafted in 1921, in that year reached the abnormal height of 78·4° F. For the 10 years previous to 1924 the average July maximum temperature at Wisley had been 71° F.; in 1923 when the disease was first noticed the average July maximum temperature was 76·3° F.; in 1924 it was only 70·6° F. and not so much damage was noticed. The average July maximum temperatures since then have been: 1925, 76·1° F.; 1926, 72·5° F.; 1927, 68·9° F.; 1928, 76·7° F.; 1929, 75·1° F. During the years 1926 and 1927 the disease was very little in evidence. The activity of the organism, as indicated by the number of new lesions observed during these years, certainly appears to be related with a temperature of over 75° F.

A culture of *B. amylovorus* was obtained from Erwin Smith through the National Collection of Type Cultures, Lister Institute, and a careful comparison made between it and the Wisley strains. There was found to be close cultural agreement between *B. amylovorus* and the Wisley strains, fermentation of sugars, non-reduction of nitrate, behaviour in milk and gelatine, optimum and maximum temperatures for growth, and thermal death points being very similar. The Wisley strains, however, always produced a green fluorescence in Fermi's and Uschinsky's solutions, and some strains also produced a slight green fluorescence in bouillon and nutrient agar. No sign of green colour was ever observed in the *B. amylovorus* cultures. The chief point of difference, however, is the arrangement of the flagella, for *B. amylovorus* has peritrichous flagella, while the Wisley strain is a *Pseudomonas* with one to five bi-polar flagella. Rosen (6) in 1926 claimed to have isolated from fire blight on apples strains of *B. amylovorus* having a single polar flagellum, but typical in all other respects. Bryan (2), however, after Rosen's paper had appeared, examined many cultures of *B. amylovorus* and found them all peritrichous. She suggested that Rosen had some other organism, and since his strains were obtained from apple lesions, there seems to be a possibility that he was dealing with *Ps. papulans* (referred to below). This difference in the arrangement of the flagella, together with the inability of the Wisley organism to attack young green pears and apples or the flowers, clearly differentiate this strain from *B. amylovorus*. While the similarities to fire blight and *B. amylovorus* are sufficiently near to call for some attention, closer resemblances are to be found in comparisons with descriptions of two other apple tree diseases. After this investigation was begun C. J. Hopkins published an account of a bacterial disease of apple trees occurring in South Africa (3). In many respects the two diseases are similar, and the causal organisms appear to be closely allied. There are, however, certain points of difference both in the development of the disease (no girdling of the main stem or branches has occurred with the Wisley disease and the extension of the diseased tissues has been in a vertical rather than in a horizontal direction), and in the cultural characteristics of the causal organism, Hopkin's organism differing from the Wisley strains in not fermenting glucose and saccharose. Hopkins noted the resemblance between the lesions on his trees, and those produced by *B. amylovorus*, but states "it became evident that the disease could not be Fire Blight, as pear trees growing in an infected orchard seemed entirely immune." This immunity is not complete with the Wisley disease, for in May 1927 out of a batch of forty young pear

trees planted close to the infected apples two died, and examination showed necrotic lesions of the cortex from which an organism identical with the apple strains was isolated. The remaining pear trees, however, remained unaffected.

Even more closely comparable with the Wisley organism is the description of *Ps. papulans* by Rose (5), the cause of a scurfy bark canker of apple trees in America. Hopkins also made a careful comparison of his strain with *Ps. papulans*, and concluded that it was a distinct species, although he agreed the two organisms were closely related. The causal organism in the Wisley disease, however, differs in no important way from *Ps. papulans*, though the scurfy bark canker has certain features which have not been recognised in the disease at Wisley, and the Wisley strains are unable to infect the apple fruit.

It is interesting to note that in a paper on apple measles, Rhoads (4) throws some doubt on the role of *Ps. papulans* as causal agent of scurfy bark, and believes that trouble to be purely physiological. Hopkins also considered the possibility that his organism was secondary, with sunscald as a primary cause. He concludes that sunscald may play some part from the fact that small cracks in the bark have been found and believed by him to be due to the sudden expansion arising from the hot morning sun playing on the frozen or almost frozen bark. Such cracks would provide a means of entry for the organism. We, also, have been in some doubt as to the true pathogenicity of the Wisley strain. The trees affected are planted in light sandy soil, and it is possible that unfavourable soil conditions play an important part in weakening the trees and rendering them susceptible to the attack of a weakly parasitic organism. (This is analogous to Briton-Jones' (1) theory that *Diaporthe perniciosa* and *Cytospora* sp. are unable to cause "die-back" of apples and pears unless the tree has become enfeebled by some physiological factor.) In support of this view is the fact that in all cases the disease appears to be caused by a closely related group of organisms rather than one fixed strain. Thus Rose isolated two distinct strains from "scurfy bark." Hopkins states that "Cultural work on the causal organism showed the possibility of the existence of several distinct strains" and the experience of the present writers confirms this view. In this connection, comparisons were made between *Ps. fluorescens liquefaciens* and the Wisley strains, some of which approached the former type. There are, however, decided cultural differences, and several attempts to induce *Ps. fluorescens liquefaciens* to produce the disease resulted in complete failure.

The authors are indebted to Mr A. N. Rawes for supplying details of the symptoms of the disease which he was the first to observe, and to Mr N. K. Gould for the photographs of the lesions. To both of these colleagues they wish to take this opportunity of expressing their thanks.

Summary.

A bacterial canker of apple trees is described and compared with cankers previously described by Rose and Hopkins, and with fire blight. The causal organism bears a certain likeness to *B. amylovorus*, but it is regarded as identical with *Ps. papulans* Rose. The suggestion is made that the latter organism should be considered as a weak parasite producing disease only where some physiological condition renders the trees liable to be attacked.

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EXPLANATION OF PLATE VII

Fig. 1. Stem of seedling apple tree, var. Premier, showing early stage of the disease. *A*. Horse-shoe shaped cracks. *B*. Blisters.

Fig. 2. *A*. Epidermis peeling off in brown flakes. *B–E*. Lesions below buds.

Fig. 3. Lesions on the internodes of an apple stem. Depressed areas surrounded by cracks.

(*Received June 27th*, 1930.)

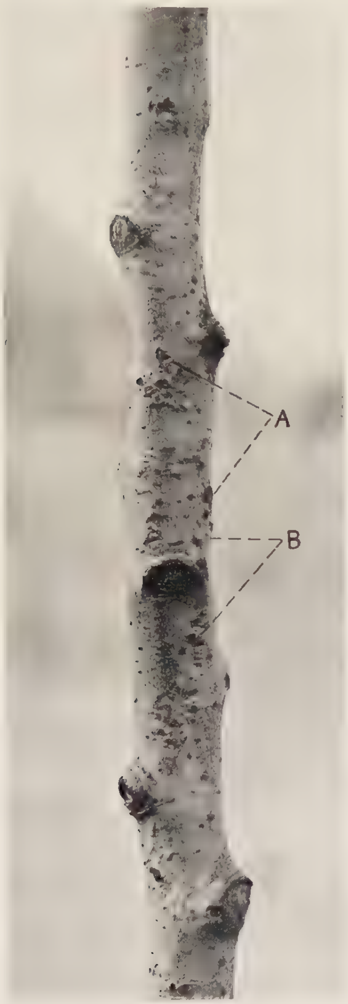

Fig. 1.

Fig. 3.

Fig. 2.

LACEY & DOWSON.—Studies in Bacteriosis (pp. 30–36).

YIELD STUDIES IN OATS

III. THE INTER-RELATIONSHIP OF THE PARTS OF THE OAT PLANT DURING DEVELOPMENT

By M. A. H. TINCKER, M.A., M.Sc.
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(With 7 Text-figures.)

CONTENTS.

I. Introduction.

This paper forms the third report, published in this Journal, dealing with the studies carried out at the Welsh Plant Breeding Station from 1922–7, to elucidate some of the factors governing the yield of oats; they were contemporary with the genetical work of other investigators, but owing to changes in the personnel of the staff have had to be curtailed somewhat prematurely.

The first paper dealt with the effect of the pre-treatment of the parent crop upon the seed produced, its germination and subsequent growth. The influence of the conditions under which the seed was harvested, stored, or graded by weight was traced upon the germination of the seed, and the growth of the seedlings in the field. The second paper took the form of a mathematical consideration, by means of correlation coefficients, of the relationship between the seed characteristics, and the early growth

of the subsequent plants of a number of samples of Record oats. The results obtained indicated that the usual germination tests gave a satisfactory indication of the field establishment; that the weight of the seed materially influenced the size of the seedlings produced during the first 10 weeks of their growth. Other points of interest were concerned with the relation between the number of plants established and their size.

In this report we consider the oat plant at a later stage of growth, employing as examples a particularly late "pure line" of the variety Ceirch du Bach and the standard variety Record. Some characteristic features of these selected varieties may be demonstrated by a brief

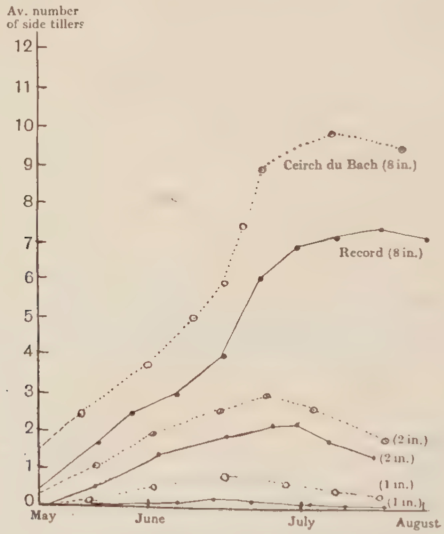

Fig. 1. Illustrating the influence of the space of the plants upon the development of the side tillers of the varieties Ceirch du Bach and Record.

consideration of a simple growth experiment, which also serves to define the general problems investigated.

Seed of these varieties, selected by weight, was sown on March 18th, 1924, in a large cereal "breeding-cage" at the following spacings: 1, 2 and 8 in. between the plants and the rows, in ground chosen for its believed homogeneity. Periodically, counts were made of the living tillers, and other notes were taken on the growth of the plants. The rate of production of the tillers is shown in Fig. 1, from which it is seen that the seasonal increase in the number of adventitious branches of the late variety exceeded that of the early variety except at the narrowest spacing. The date of the

emergence of the first panicles of the main shoots of the plants grown under these conditions was:

Space	1 in.	2 in.	8 in.
Record	July 9th	July 11th	July 13th
Ceirch du Bach	July 15th	July 16th	July 25th

The influence of the increased space caused the production of panicles to be delayed, the later variety being more susceptible to this factor. The behaviour of the main axis was not totally independent, for the number of side branches formed influenced the rate of development of the primary shoot.

One is therefore confronted with the problem of the relationship existing between the different tillers of the plant and the general rate of development. The investigations here reported form an attempt to elucidate further this relationship.

II. Method of experimentation.

An experimental method was chosen in preference to a statistical consideration of the behaviour of more normal plants in the field, for it enabled a wider latitude of spacing conditions to be employed, and also enabled parts of the plant to be removed when required. Forty-eight plots (800 plants in each plot) were sown at three spacings: 1, 2 and 8 in. At various stages of development the plants of the various plots were treated thus:

(1) The side tillers were cut off.

(2) From other similar plants the main axis was removed at the same date.

(3) Controls.

The subsequent growth made after cutting the plants was estimated by obtaining dry weight data after periods of different length. The roots were carefully dug up, washed, and weighed. Many of the smaller fibrous roots were undoubtedly lost, but the larger roots enabled a rough estimate of the general root development to be obtained, so that it was possible to observe some effects of the cutting treatment of the "tops" on the roots. The plants were removed from the plots, washed, then separated into their component parts, and dried in an oven at 85° C. to constant weight. The figures shown in the tables are average figures obtained from samples of *at least* eighty plants weighed separately; where the incidence of pests or disease did not necessitate the discarding of any plants at all, then the average figures were obtained from more than 170 plants. Any insect or other damage to a plant caused the discarding of that particular plant and all its immediate neighbours.

Data relevant to our discussion are shown in Table I (Record) and Table II (Ceirch du Bach), and in Figs. 2–7.

Table I. *To show the effect of cutting the tillers of oats upon the subsequent development of the plants. Variety Record. Sown April 14th.*

(1) Cutting treatment	(2) Space of plants (in.)	(3) Date of cutting	(4) After 3-week periods of growth	(5) Dry wt. 100 plants main axis (gm.)	(6) Dry wt. 100 plants side tillers (gm.)	(7) Dry wt. 100 plants roots (gm.)	(8) Dry wt. 100 plants total (gm.)
Main axis cut	8	9. vi.	8th–11th	—	136·6	24·8	161·4
Side tillers cut	8	,,	,,	87·1	58·1	36·8	182·0
Control not cut	8	,,	,,	73·0	47·8	39·2	160·0
Main axis cut	8	23. vi.	11th–14th	—	90·2	46·8	137·0
Side tillers cut	8	,,	,,	178·4	33·2	48·7	260·3
Control not cut	8	,,	,,	148·0	159·5	53·8	361·3
Main axis cut	2	9. vi.	8th–11th	—	38·8	12·7	51·5
Side tillers cut	2	,,	,,	97·5	7·2	29·3	134·0
Control not cut	2	,,	,,	90·8	8·6	28·6	128·0
Main axis cut	2	23. vi.	11th–14th	—	65·0	17·2	82·2
Side tillers cut	2	,,	,,	143·8	0·95	27·7	172·5
Control not cut	2	,,	,,	134·1	9·9	23·5	167·5
Main axis cut	1	23. vi.	11th–14th	—	8·3	5·0	13·3
Side tillers cut	1	,,	,,	57·9	0·4	7·3	65·6
Control not cut	1	,,	,,	58·3	0·8	6·6	65·7

Table II. *To show the effect of cutting the tillers of oats upon subsequent development. Variety Ceirch du Bach.*

(1) Cutting treatment	(2) Space of plants (in.)	(3) Date of cutting	(4) After 3-week periods of growth	(5) Dry wt. 100 plants main axis (gm.)	(6) Dry wt. 100 plants side tillers (gm.)	(7) Dry wt. 100 plants roots (gm.)	(8) Dry wt. 100 plants total (gm.)
Side tillers cut	8	23. vi.	11th–14th	82·8	51·2	21·8	155·8
Control not cut	8	,,	,,	125·6	231·8	49·0	406·4
Special manured plot							
Side tillers cut	8	23. vi.	11th–14th	189·8	215·9	81·9	487·6
Control not cut	8	,,	,,	164·6	634·4	63·0	862·0
Main axis cut	2	9. vi.	8th–11th	—	60·8	15·4	76·2
Side tillers cut	2	,,	,,	58·4	31·2	23·4	113·0
Control not cut	2	,,	,,	48·9	29·6	25·8	104·3
Main axis cut	2	23. vi.	11th–14th	—	82·4	29·5	111·9
Side tillers cut	2	,,	,,	108·4	13·5	30·9	152·8
Main axis cut	1	23. vi.	11th–14th	—	16·3	3·7	20·0
Side tillers cut	1	,,	,,	56·3	2·1	4·4	62·8
Control not cut	1	,,	,,	46·4	2·5	6·6	55·5

III. Data obtained.

(*a*) *Record.*

(*a*) At the wide spacing the plants from which the main axis was removed 8 weeks after sowing rapidly recovered, so that even after such drastic treatment the increased growth of the side tillers caused the total

dry weight of the plants to equal approximately that of uncut controls. Despite this the root development suffered; as it did also, but to a smaller extent, when the side tillers were removed. Those plants from which the side tillers were cut off, formed further side branches and new leaves so that after 3 weeks the weight of the newly formed side tillers closely resembled that of side tillers on previously uncut plants. Such plants recovered so rapidly at this stage of their growth that they were (as heavy or) heavier than the controls.

Moreover, the main axis of these plants from which the side tillers were removed developed more rapidly.

At a later stage of growth (11 weeks) when the main axis was cut off from a further sample of similarly spaced plants they did not recover so rapidly, so that at a subsequent date they were much lighter in weight than the controls, despite the subsequent increased rate of growth of the side tillers. The removal of the side tillers at this later stage also caused a decrease in the subsequent weight, despite the fact that the main axis grew more rapidly. At this later stage of growth (11 weeks old) the removal of the main axis did not have such a marked effect upon the root development as at an earlier date, but the cutting of either the main or side tillers caused a decreased rate of root growth.

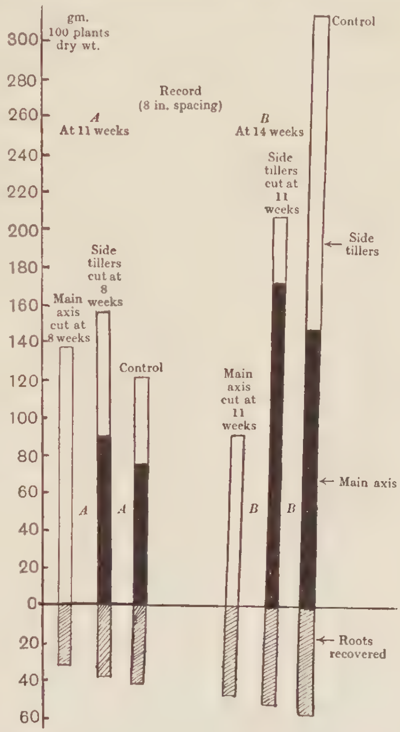

Fig. 2. Illustrating the effect of cutting off the main axis, or side tillers, of widely spaced plants of Record oats at 8 and 11 weeks. The dry weights were obtained 3 weeks after cutting.

(*b*) At the medium spacing (2 in.) the plants from which the main axis was severed at 8 weeks after sowing did not recover; their subsequent dry weight, when estimated 3 weeks after cutting, was less than half that of the controls. Plants from which the side tillers were removed were slightly heavier than the uncut controls; again it was observed that the removal of the side tillers caused an increased rate of growth of the main axis. The root development was checked by the removal of the

main axis, but appeared as strong in those plants from which the side tillers were cut off as in the controls. It must, of course, be remembered that in these plants the ratio by weight of the main axis to the side tillers was very much greater than in the case of the plants enjoying the widest spacing conditions. At an interval of 8 in. this ratio was 1·6/1, whilst at an interval of 2 in. between the plants the ratio was 10·6/1, or about six times as great.

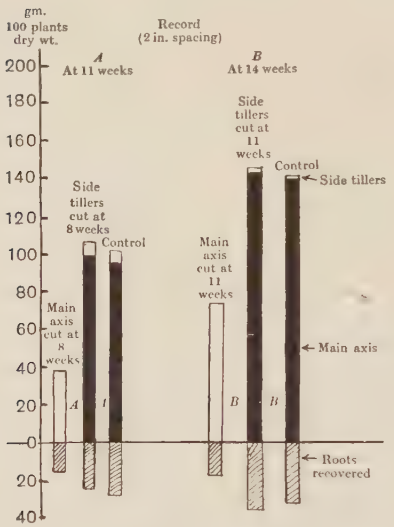

Fig. 3. Illustrating the effect of cutting off the main axis, or side tillers, of spaced plants of Record oats at 8 and 11 weeks. The dry weights were obtained 3 weeks after cutting.

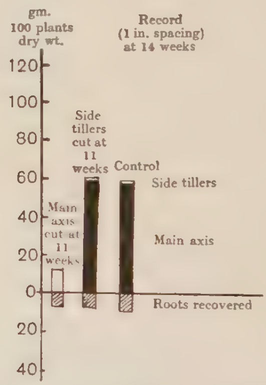

Fig. 4. Illustrating the effect of cutting off the main axis, or side tillers, of crowded plants of Record oats at 11 weeks. The dry weights were obtained 3 weeks after cutting.

The data obtained from the second date of cutting showed that the removal of the main axis greatly injured the development of the plants from the eleventh week of their growth. At this spacing the removal of the side tillers so late in the development of the plants did not cause any serious decrease in the subsequent weight, but little new growth of lateral branches took place, the main axis grew slightly more rapidly however.

(c) At the very close spacing where little branching took place, the removal of the main axis naturally caused a very marked decrease in the subsequent weight of the plants; the rate of recovery of these plants was too slow to be appreciable in the limited time available before they were removed from the plots. The removal of the side tillers, which were very

small, caused little or no difference in the growth rate of the plants. At this very close spacing the ratio by weight of the main axis to the side branches was very high, being about 74/1.

(*b*) *Ceirch du Bach.*

(*a*) At the wide spacing (8 in.) the plants from which the side tillers were cut off at 11 weeks recovered moderately quickly, so that the new

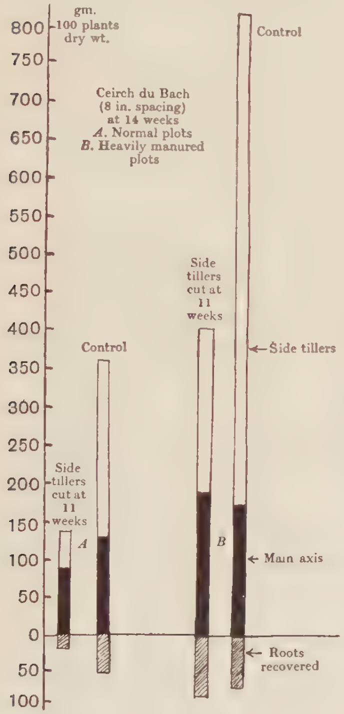

Fig. 5. Illustrating the effect of cutting off the side tillers at 11 weeks from widely spaced plants of Ceirch du Bach oats growing on a normal and heavily manured plot. The dry weights were obtained 3 weeks after cutting.

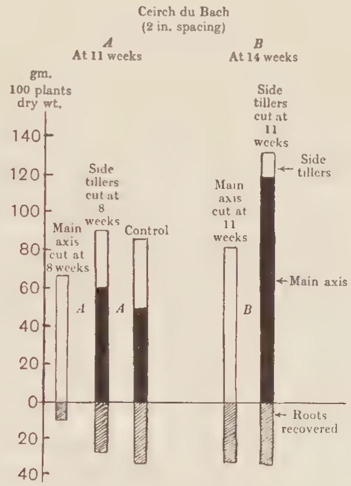

Fig. 6. Illustrating the effect of cutting off the main axis, or the side tillers, from spaced plants of Ceirch du Bach oats at 8 and 11 weeks. The dry weights were obtained 3 weeks after cutting.

growth made after the cutting contributed a fairly large weight to the subsequent total. The roots of the plants from which the side tillers were cut off did not develop so rapidly as those of the controls. A further series grown on a manured plot, the soil of which had received a liberal

dressing of a complete fertiliser a year previously, gave results of a slightly different nature. The original plants were naturally larger; after cutting, the rate of recovery was more rapid, so that the subsequent weight of the cut plants, as compared with the controls, was greater. Under these conditions there was also no apparent persistent damage to the root system—in fact the roots obtained from the "cut" plants were actually heavier than those from the controls. At the wide spacing the ratio of the main axis to the side tillers was 1/2 on the normal soil and nearly 1/4 on the well-manured plot.

(*b*) At the medium spacing (2 in.) the plants from which the main axis had been cut off at 8 weeks did not recover so rapidly, and were subsequently lighter in weight than the controls. The root development was checked by the removal of the main axis, but appeared to be almost as strong in those plants from which the side tillers were cut off as in the controls. At this spacing the ratio of the main axis to the side shoots was nearly 2/1, showing a relative decrease from that at the wide spacing of about eight times. The removal of the main axis at a later date (11 weeks) did more damage to the subsequent growth of the plants than did the removal of all the side tillers.

(*c*) At the very close spacing (1 in.) the removal of the main shoot caused much damage to the plants from which they did not recover. The cutting off of the side tillers caused little or no damage to the plants. Here the ratio of the main axis to the side shoots was about 23/1.

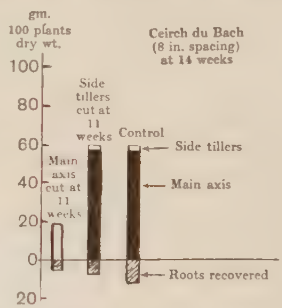

Fig. 7. Illustrating the effect of cutting off the main axis, or side tillers of crowded plants of Ceirch du Bach oats at 11 weeks. The dry weights were obtained 3 weeks after cutting.

(*c*) *Comparison of the varietal responses.*

In comparing the data of the two varieties it was observed, at the wide spacing, that the rate of recovery after the side tillers had been cut off from plants 11 weeks old, was, relative to the uncut controls, more rapid in the variety Record than in the variety Ceirch du Bach. The latter variety normally forms more side tillers. Evidently the greater weight of tillers removed represented a high proportion of those that the plant could form, and this more slowly growing variety could not make as much *new* growth as the variety Record, which normally forms a smaller number of tillers. At the medium spacing when the main axis was cut

off, the multi-tillering variety Ceirch du Bach was apparently at an advantage, the subsequent growth made by the side branches brought the total up to three-quarters of that of the controls, whilst in the case of the variety Record the subsequent growth was less than one-half of that made by the controls. In every case, with both varieties, the removal of the main axis caused more damage to the roots than did the cutting off of the side tillers.

In seven out of the eight cases considered the removal of the side tillers caused an increased growth of the main axis. Similarly the removal of the main axis resulted in the more rapid growth of the side branches of both varieties.

IV. Consideration of the results.

(a) *Root development in its relationship to shoot growth.*

With our technique in the field it was not possible to recover all the fine roots or all the roots growing at the deeper levels. Yet by carefully washing away the soil all the larger roots, and particularly those in the first foot of soil, were recovered, so that it was possible to obtain data of value and interest. It must be remembered that the soil in which our plants grew was a shallow one overlying a shale-like rock and, moreover, the general farm practice in Wales often includes the growing of this crop upon soils of even less depth—of but 8 or 9 in. in the hill districts.

When the weights of the control or uncut plants are considered, it is seen that the weight of the top growth compared with that of the *roots recovered* increases with the age of the plants. In the case of the plants at 8 and 2 in. intervals this ratio—tops recovered/roots recovered—rises from 4/1 approximately to 6/1 between the eighth and eleventh week. At the very close spacing of 1 in. the ratio attained the level 9/1 at 8 weeks, which clearly illustrates the effect of the limited space upon the rate of development of the roots. Whereas the development of the roots in the first foot of the soil had apparently reached its maximum at 8 weeks, with plants enjoying wider space further development of the roots near the surface continued until 11 weeks.

It may be that the morphological structure was reduced or curtailed by limited space; or it may well be that the physiological organisation, into conducting and absorbing zones, was altered. It appears that a zone, normally a conducting zone, can again quickly assume the functions of absorption, for it has been repeatedly observed that on breaking the roots of large plants, by plucking them up, new root hairs are rapidly developed on old and comparatively thick roots. It might well be that

some such similar modification of the usual organisation takes place either under the unnatural conditions of little space or when the plants were cut. For these and other reasons it is not possible to make the unqualified assumption that the dry weight data represent the relative efficiency of the root systems so compared.

The following points were observed with the variety Record. The removal of the main axis from plants grown at 8 in. interval when 8 weeks old caused a serious decrease in the rate of the root development amounting to about 33 per cent. When the ratio tops/roots was estimated 3 weeks later, the very rapid rate of recovery of the tops caused this ratio to be greater in the cases of the previously cut plants. Also, at the spacing of 2 in., a very serious check to the development of the roots amounting to about 50 per cent. was produced by cutting the main axis. The rate of subsequent recovery in this case was such that the ratio tops/roots was slightly smaller than that of previously uncut plants. The removal of the side tillers at this stage of growth resulted in little diminution of the rate of root growth, and the ratio tops/roots subsequently estimated was slightly greater than that of uncut plants.

At 11 weeks the cutting of the main axis of plants growing at both 8 and 2 in. intervals produced more lasting effects, so that the ratio tops/roots, when estimated 3 weeks later, was considerably reduced compared with that of previously uncut plants. The root system had not suffered as acutely as it did by the earlier cutting of the tops. The removal of the side tillers similarly caused a decreased ratio tops/roots to be observed 3 weeks later in the case of plants growing at 8 and 2 in. intervals; such treatment caused little damage to the root system.

With this variety therefore it appears that the early removal of the main axis damages the root system, although the tops recover well. Cutting at a later date caused comparatively little damage to the root, but the rate of recovery of the tops was very much slower.

The following points of interest were obtained from the data obtained with the variety Ceirch du Bach. At the wide spacing of 8 in. the influence of the previous season's heavy application of manure to special plots was observed in a ratio of tops/roots of twice the magnitude of that obtained on the standard plots. On the standard plots with plants grown at the widest spacing (8 in.), the effect of cutting side tillers at an early date was to damage severely the root growth, and also to cause a reduced ratio tops/roots when estimated 3 weeks later.

At the spacing of 2 in., cutting the main and side tillers at 8 weeks reacted adversely upon the subsequent development of the root system.

But the subsequent rate of recovery of the tops was such that the ratio tops/roots was greater than that of the uncut controls, when estimated 3 weeks later. Such results are due to the fact that, at the wide spacing, the development of the side branches was more advanced and, in consequence, more side tillers were cut off than at the closer spacing.

Table III. *The influence of cutting the tillers on the subsequent growth as measured by the ratio tops/roots (dry weight). Date of sowing, April 14th.*

Variety	Space of plants (in.)	Tillers cut	Date of cutting	Ratio tops/roots (3 weeks later)
Record	8	Main axis	June 9th	5·5
,,	8	Side	,,	4·0
,,	2	None	,,	3·1
,,	2	Main axis	June 23rd	2·0
,,	2	Side	,,	4·4
,,	2	None	,,	5·7
,,	2	Main axis	June 9th	3·1
,,	2	Side	,,	3·6
,,	2	None	,,	3·5
,,	2	Main axis	June 23rd	3·8
,,	2	Side	,,	5·2
,,	2	None	,,	6·1
,,	1	Main axis	,,	1·6
,,	1	Side	,,	8·0
,,	1	None	,,	9·0
Ceirch du Bach	8	Side	,,	6·3
,, ,,	8	None	,,	7·3
,, ,,	2	Main axis	June 9th	4·0
,, ,,	2	Side	,,	3·8
,, ,,	2	None	,,	3·1
,, ,,	8*	Side	June 23rd	5·0
,, ,,	8	None	,,	12·7

* Manured plot.

(*b*) *Some after-effects of the early cutting of the tillers.*

At a later date it was possible to observe the effect of the cutting treatment on the growth of the flowering branches. This was estimated by noting the date of emergence from the leaf of the first panicle on the earliest 33 per cent. of the plants. With the variety Record the effect of cutting the main axis off on June 9th and on June 23rd was to delay the date of panicle emergence by 9 and 11 days respectively. The removal of the side tillers on these dates caused the panicles of the main axis to be 2 days earlier, at the wide spacing.

The removal of the main axis of plants spaced at 2 in. also delayed the production of panicles; cutting the side tillers accelerated the emergence of the panicles of the main axis by 2 days.

At the close interval of 1 in. the delay caused by the removal of the main axis was much greater amounting to several weeks.

With the variety Ceirch du Bach at the wide spacing (8 in.) the removal of the main axis on June 23rd caused a delay in the time of panicle emergence of 3 weeks, both on the normal plots and on the specially manured plot. Cutting the side tillers off once from plants grown at 8 and 2 in. did not make the date of panicle emergence appreciably earlier. The side tillers of another series[1] were cut off at weekly intervals; with both varieties this caused the main axis to develop more rapidly in the plants spaced at 8 and 2 in. This effect was not observed at the closest spacing of 1 in., where very little material was removed. It is concluded, from the evidence available, that almost *complete removal* of the side tillers is necessary to accelerate the development of the main axis. After fertilisation, and whilst the grain was ripening, the differences observed previously in the rate of development of the tillers due to the earlier cuttings was still visible.

With the variety Record grown at 2 in. the plants, from which the side tillers were cut off in early June and those cut at weekly intervals, were slightly earlier than the uncut controls when the latter were at the "early dough" stage and the paleae beginning to turn yellow. At the wide spacing (8 in.) similar differences were also noticed, but at the closest spacing (1 in.) no difference was seen.

With the variety Ceirch du Bach at 8 in. those plants from which the side tillers were cut off once began to show yellow coloration earlier than did the controls. At 2 in. those plants cut in June also ripened earlier than the controls, but those cut weekly seemed to have suffered irreparable damage and were considerably later. In this connection it is pertinent to point out again that the weight of the tillers cut off at weekly intervals from the two varieties differed greatly; far more was cut off from Ceirch du Bach.

V. General discussion.

The data presented serve to emphasise the plasticity of the cereal plant. The ratio of the parts varied within wide limits, and whilst various treatments caused visible changes in the organisation of the sub-aerial growth, these changes are also reflected in the rate of the development of the root system. It remains to present a rational explanation, as far as it is possible to do so, of the data considered in this paper.

[1] Data not shown in tables.

In theory, but not in practice, it is possible to distinguish three phases in the life history of the branches (including the main axis) of the plant. The first phase, a "developmental" one, starts with the initiation of the primordia of the branch, or, in the case of the main axis, with the young embryonic shoot, and includes all stages of growth until the first leaf (or leaves) functions and produces carbohydrates. It must be remembered in this connection that the leaf develops chlorophyll some time before it has attained the active photosynthetic condition. During this phase the branch is dependent upon the parent plant for its supply of food (the main axis upon the seed). Whilst it is not possible to determine precisely when the rate of photosynthetic activity is such as to meet the needs of the shoot, this stage marks the beginning of the second or "vegetative" phase. It appears that the main axis is capable of supporting further growing centres at a fairly early stage in its own development. During the vegetative phase there is presumably an excess of manufactured carbohydrates over immediate requirements of the individual shoot and translocation occurs to the roots and other centres, including the developing side shoots. The "final phase" is either associated with the production of a panicle and the subsequent ripening of the seed, or, on the other hand, the more rapid decline of the active leaf area and the death of the shoot. In the former event the elongation of the internodes and of the leaf sheaths as well as the formation of the flower parts utilise, at least part of, the accumulated and manufactured carbohydrates. Also during this early flowering period the respiratory activity is high, and there would seem to be definite indications that the plant is then particularly sensitive to the external conditions. In the event of the death of the tiller it may be assumed that some of the compounds in its tissues are translocated to the surviving branch (or branches) before complete decay.

With plants growing at intervals of 2 in. the damage caused to the roots by cutting the main axis when 8 weeks old may be due to the decrease in the available carbohydrates brought about by reducing the leaf area, for the main axis was then cut in the "vegetative" phase of its growth. The side tillers were in the early "developmental" phase, and by cutting them off no damage to the root system was noticed 3 weeks later. At an older age the removal of the side tillers produced a deleterious effect upon the subsequent root growth; such tillers were removed when they were in the "vegetative" phase and were presumably actively engaged in photosynthesis.

With plants growing at 8 in. the side tillers were larger at the early

date of cutting and were about to enter the "vegetative" phase of their growth, so that the rate of development of the root system was reduced when they were cut off. The complete removal of the side tillers, however, was associated with an increased rate of growth of the main axis, indicating perhaps that centres of food utilisation, formed at the expense of the main axis, were removed. As the leaf development was greater on the side tillers at the wide spacing, the removal of the main axis caused a smaller reduction in the rate of growth of the roots of these plants when compared, proportionally, to that caused by the removal of the main axis from plants growing at only 2 in. interval. This is confirmed by the observations made by cutting the main axis at a later stage—the proportional reduction in the root system under the conditions of wide space as contrasted with the reduction at closer intervals was even smaller than that observed at the earlier date.

Briefly, the effect of cutting the tillers upon the development of the roots varied with the stage of the tillers cut; cutting small developing tillers actually resulted in an increased rate of root development, whilst the removal of tillers in their vegetative condition resulted in a retardation of the root growth.

Turning to a consideration of the inter-relationship of the parts of the sub-aerial growth, it has been shown that the removal of side tillers whilst they are in the "developmental" phase accelerates the rate of growth of the main axis; this is thought to be due partly to the fact that the drain upon the food supply of the main axis is almost completely terminated by such treatment. At the wider spacing there are more such centres, and the acceleration caused by their removal is more rapid than at the closer space. With the variety Ceirch du Bach, which possesses the ability of forming many branches, it is necessary to remove all the young developing branches to observe rapid acceleration of the growth of the main axis; otherwise the effect is masked by the formation of new side branches.

The removal of the side or main axis of plants with a well-developed root system may well cause the rate of supply of soluble mineral metabolites to the remaining tissues to be increased. Whilst it was repeatedly observed that the remaining tissues grew faster than similar parts on an uncut plant, no evidence was obtained to substantiate the view that *an excess* of nitrogenous compounds, as indicated by prolonged vegetative vigour, accumulated in the remaining tissues. Such a prolonged period of vegetative vigour was not observed either in the case of plants growing on the standard plots or on the manured plots. The floral development of

the main axis, where it remained, was accelerated by cutting the side tillers.

There would appear to be a considerable mass of available data in the literature, indicating that, as the cereal plant develops, the ratio carbohydrate/nitrogenous compounds increases in the plant, but as yet we do not know which carbohydrate compounds are the most important for the production of the flower primordia. Presumably the celluloses and similar carbohydrates are eliminated, as they are unlikely to be hydrolysed.

Our results indicate that plants enjoying wide space and having received no artificial check to their growth flower slightly later than plants growing closer together. Presumably, on this hypothesis, the ratio C/N rises more slowly, yet the removal of the side tillers caused the main axis to flower more rapidly, so that by such a hypothesis we must suppose that the ratio C/N rose more rapidly; which is to say that any increased rate of gain in nitrogenous compounds was more than compensated for by the decreased drain of the carbohydrates brought about by cutting away developing side tillers.

The agronomic bearing of such considerations is related to the yield of straw obtained from a given variety. It has been shown by one of us (M. G. J.) that the yield of straw is closely correlated with the length of the growing period, so that the winter and late ripening varieties produce very much more straw than do the spring or early varieties. These straw-yielding types produce many tillers in their early stages. If each tiller in its young stage acts as a drain upon the carbohydrates, then the ratio C/N would tend to rise slowly, so that the production of flowers would be later than in the case of a variety sown at the same time forming only few tillers. The early varieties of which the American "60-day" serves as an example *do* form few branches, and in them the carbohydrates are presumably "concentrated" in the main axis, which rapidly reaches the flowering condition. Such varieties yield but little straw. It has been repeatedly observed that the varieties most suited to the hilly districts of Wales where the "season is late" and where the soil is often poor are those which form many tillers. This habit tends to ensure an even stand, despite irregular germination and establishment, and the plants mature slowly to the flowering stage. However, few tillers survive. Possibly the total sum of the compounds translocated to the surviving tillers from those that die early is of importance to the surviving parts of the plant. It is, therefore, an interesting though speculative suggestion that, under these adverse conditions of growth, the successful varieties owe their success, amongst other reasons, to the high death-rate of the side tillers,

and the subsequent "concentration" of the food products in the surviving main axis. Unfortunately no data, by which such a hypothesis can be tested directly, are yet available.

The data presented in this paper tend throughout to emphasise the importance of considering the oat plant as a unit rather than as a complex collection of branches of semi-independent units, for the development of one part has been shown to react upon the growth of another part, and to leave a lasting effect upon the rate of development of the whole plant.

VI. Summary.

1. This paper is the third report of a series dealing with the oat crop, and is concerned with the inter-relationship of the developing parts of the plant in the field.

2. Experiments are reported in which the various component parts of the sub-aerial growth of spaced plants growing at stated intervals were removed at successive stages of development. The after-effect of such treatment was observed upon the rate of growth of both the roots and shoots.

3. Whilst the removal of lateral branches in their developmental stages, during which they are dependent upon the parent branch, resulted in an acceleration of the rate of growth of the parent axis, the removal of any part of the sub-aerial growth which is actively engaged in photosynthesis caused a reduction of the rate of growth of the plant as a whole. Such changes in the rate of growth are reflected in the root system and the ratio of root to shoot.

4. The influence of the removal of part of the shoot system is a lasting one. It can be traced out upon the time of flowering, and its effect noticed upon the rate at which the remaining parts ripen.

5. These considerations are closely related to the previous agronomic observations dealing with the relative yield of grain and straw obtained from a variety. The late, multi-tillered varieties, are the straw yielders *par excellence*. In them the relationship of the parts of the plant more closely corresponds to that observed under conditions of wide space than under limited space. The early varieties in general behaviour more closely resemble the plants grown at close intervals and those plants grown at wide intervals from which the side tillers were removed.

The authors desire to express their thanks to Prof. R. G. Stapledon, the Director of the Welsh Plant Breeding Station where this work was

carried out, for so kindly providing the facilities necessary, but more particularly are they indebted to him for his enthusiastic interest and the encouragement given at all stages. To the members of the technical staff we are indebted for assistance in the cutting, counting, and weighing of the large number of plants.

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PASTURE PRODUCTION IN AREAS LIABLE TO INFECTION BY *ONCOPERA INTRICATA* (WALKER) IN TASMANIA

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As a result largely of economic pressure and the need for higher efficiency on the holding, the pasture problem in Tasmania has become rather more pressing than heretofore. A considerable factor in the maintenance of a pasture sward is the incidence of the grubs of various insects which attack the grass. Most important of these undoubtedly is the underground type characterised in Tasmania by the underground grass grub. The practical man possibly includes under this common name more than one species, but it may be safely assumed that this in large part refers to the larva of a Hepialid moth, *Oncopera intricata* (Walker). There is no doubt that *O. intricata* is widely spread throughout the island state and that considerable damage is done by it to pastures, but no precise knowledge is available. The nature of the damage to the individual pasture plants has been well described by Hill(1) who states that on large partially cleared and on uncleared grazing areas the pest is so serious that any attempt at pasture improvement is vain.

On the second and third classes of grazing land which are usually held in small and medium sized blocks, perhaps too hilly to cultivate, but capable of giving good yields of grass, useful herbage is eliminated by the grub, often in conjunction with rabbits. The result on such areas is the reversion to bracken. Arable areas laid down to pasture may be completely denuded of all profitable herbage and recovery of the pasture is uncertain.

In the case of first-class land which is usually held in small and medium sized blocks, dairying and mixed farming is practised. There the pastures are of a more "temporary" character than in the areas mentioned above. In these cases, pastures of more than 2 years' duration may be denuded, and either another arable crop introduced or the pasture re-sown.

[1] Formerly Superintendent of Research, Tasmania.

No real economic control of the pest has so far been devised. The life history has been clearly stated in detail by Hill, and may be briefly indicated here. The eggs are laid about February to March, being deposited in batches in the shelter of a tussock. The egg stage in nature probably extends for about 63 to 70 days. The larvae, after hatching, live in a community, and almost immediately attack young grass in the near vicinity. The larva constructs a covered way and, later, a burrow in the soil. Feeding is by night, and the area attacked by each individual caterpillar is being constantly extended. Damage consists in the cutting off at the base of the shoot of the grass plant. Whole plants are denuded of the shoot at or above the ground level, so that eventually they die.

The writer, preparatory to embarking on work on possible controls or checks to the grass grub, put the following questions to practical graziers and pastoralists. The questions were sent to all individuals whose names appeared on a complete list maintained by the State Department of Agriculture for regulatory purposes. In addition, the questions were sent to the council clerk of each municipality. The whole state was thus covered. The questions asked were:

1. Does grass grub occur in your station?
2. Is the infestation severe?
3. At what season of the year does the damage appear?
4. How long does the grub remain alive?
5. Do the infested pastures recover to any extent?
6. If so, when?
7. Which type of pasture is most seriously attacked:
   (*a*) Artificial or native grass?
   (*b*) Hill land or flats?
8. How far do you consider the stock carrying capacity of the pastures is reduced?
9. Remarks.

The number of replies received was 191. Carefully summarised the replies may be shown as follows:

1. *Occurrence of grub.* Practically universal. Affirmative replies numbered 184 (nearly $96\frac{1}{2}$ per cent.). Of the seven negative replies, three were from non-pastoral districts.

2. *Severity of infestation.* Less than 25 per cent. of those who sent affirmative replies never suffered severely. The majority of the remainder report serious infestation at times, chiefly in dry seasons. A number of pastoralists state that the grub is the most serious pest with which they have to contend.

3. *Season at which damage appears.* In the spring (from August to the end of the year) according to the great majority of replies. In a few cases the damage appears in July and even earlier, while in others it does not appear till January.

4. *Time that the grub remains active.* Usually from 2 to 4 months. Unless checked by rain in excess it remains active during the period of greatest growth of grass. A few replies mention the hardening of the soil due to drying out as a possible limiting factor of activities.

5. *Recovery of infested pastures.* There is much vagueness in reference to this. Some replies state, "Yes, if re-sown," others, "In two or three years." A large number hold that a fair or complete recovery follows a good rain. "Artificial" grasses are, in many instances, stated to be killed right out and their place taken by dandelions, thistles, etc. Pastures of native grass appear to recover more quickly and completely, though a few replies indicate exceptions to this. A few returns showed a definite negative to this question, while many stress the fact that recovery is only partial.

6. *Time of recovery.* See Section 5. There are great differences of opinion. The most frequent replies are "After rain," "End of year," "Following Autumn," and "Following Spring." It seems to be to some extent a question of the nature of the season.

7. *Types of pasture affected.*

(*a*) Nearly 60 per cent. of the replies state "Artificial Grass," less than 10 per cent. "Native Grass," most of the remainder "Both." English rye-grass seems to suffer most. Many say that clovers are immune, particularly subterranean (*Trifolium subterraneum*). Others have found clover affected and in one instance, subterranean clover is said to have been taken. According to some replies, cocksfoot grass, shown by Hill to be severely attacked, is little affected, while couch is usually free.

(*b*) Hilly land seems to be affected more than flat, but the nature of many replies indicates that much depends on the amount of moisture in the soil, dry flats often being badly infested. The grub seldom appears in land that has been flooded. All types of soil appear to suffer, light or loamy soil being most frequently mentioned.

8. *Reduction of stock-carrying capacity.*

45 estimate it at less than 25 per cent.
46 ,, ,, 25 to 50 per cent.
70 ,, ,, 50 to 100 per cent.

9. *Remarks.*

(*a*) *Conditions favourable to grub.*

(1) Dry winter and spring.

(2) Good growth of grass previous season.

(3) Old grass left in autumn harbours grubs or eggs.

(4) Country newly opened up. (Three replies.)

(*b*) *Control measures.*

(1) Flooding irrigable land.

(2) Close-grazing.

(3) Top-dressing.

(4) Light harrowing to expose grubs to birds.

(5) Encouraging birds, especially plovers and starlings.

(*c*) *Miscellaneous.* A few consider that the grub benefits the soil by ventilating it and allowing moisture to penetrate. While in some instances top-dressing is recommended, some farmers consider that top-dressing is useless unless the grub has been previously eliminated. Native grasses are being affected more than formerly. Bandicoots were mentioned in two replies as grub destroyers. The pest practically compels tillage of infected pasture lands.

It may be assumed from these replies that the grub may appear at any point in Tasmania where conditions of soil and climate are in any way favourable to the pest. The damage rate is high and comes with the flush of grass when the stocking should be at its maximum; thus the pressure on the pasture of both stock and pest are both at a maximum simultaneously.

The extent of the damage, as shown by the replies to Question 8, must not be stressed over-much, as infection may not be continuous over the whole area. The estimates given, however, show, at the very least, that the grub must be countered if economic animal production is to be carried on.

The replies to Question 9—an invitation to remark on the pest—indicate possible points in management which may be useful in some areas. One, certainly, is important—the elimination of old grass by the time the moth oviposits.

Top-dressing, as an answer or partial answer to the grub attack, must depend on something being left to grow during and after the grub's period of activity.

A considerable amount of observation of the residuum left after severe grass grub attacks in various districts indicated a possible counter to

the pest. Apart from species not eaten by the grub (usually not grasses), the only types leaving a nucleus for quick recovery were grasses with some mechanism such as stolons and rhizomes for vegetative propagation. The pest did not consume such vegetative structures though the aerial leaves might be treated in the usual way.

The vagueness of the replies to Question 5 indicate that the practical pastoralist has noted the possibility of recovery, though perhaps not realising the underlying reason for the variability of return.

The replies to Question 7 are also important, stressing as they do the vulnerability of "artificial" grasses. Artificial grasses in Tasmania up till lately meant in large part rye-grass and cocksfoot species with little or no vegetative vigour. Unfortunately the use of grass species with mechanisms for vegetative propagation has a number of drawbacks. Firstly, the produce of such species is usually poor, both as to quality and quantity, and much of the food manufactured is stored in the reproducing organs and these are usually out of the way of the grazing animal. From these considerations only it would seem that the production of pasture by species of the type advocated would not be profitable on first-class land. On land not remunerative to the plough, or owing to steepness or roughness, production of such pasture, though not of first quality, would probably render them economic in face of grub attack. Further, species propagating vegetatively are usually more or less bad weeds of arable land and, therefore, their use is not advocated for first-class land, but in the latter case temporary pasture is possible and grub attack may in this way be countered. The use of annuals which re-sow themselves each year may also act as a counter to the grub and the reaction of subterranean clover (*Trifolium subterraneum*) in this regard is important.

The questionnaire was sent out by the writer while a member of the Staff of the Department of Agriculture, Tasmania, and grateful acknowledgment is made to Mr W. Whitham of that department for assistance in tabulating the replies, and also to the pastoralists and council clerks who replied.

Summary.

The replies to a questionnaire sent out to a large class of sheep owners and pastoralists in Tasmania indicate: (1) that *Oncopera intricata* (Walker) is a pest of first-class economic importance in the State of Tasmania, precluding as it does successful pasture production over large areas of the country; (2) that grasses with a mechanism for natural

vegetative propagation, *e.g.* stolons, etc., are likely to persist through a severe attack and by their recovery after the pupation of the grass-attacking larvae provide some return rather than allow the ground to go out of production or be covered by unprofitable weeds; (3) the replies plus external evidence show that a sward of creeping grasses and clovers will be likely to be most useful.

REFERENCE.

(1) HILL, G. F. (1929). The Tasmanian grass grub (*Oncopera intricata* Walker). *Bull.* 11, *Commonwealth Council of Australia for Scientific and Industrial Research.*

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THE PRODUCTION OF ETHYL ALCOHOL AND ACETALDEHYDE BY FRUITS[1] IN RELATION TO THE INJURIES OCCURRING IN STORAGE

PART II. INJURIES TO APPLES AND PEARS OCCURRING IN THE PRESENCE OF OXYGEN AND IN THE ABSENCE OF ACCUMULATIONS OF CARBON DIOXIDE IN THE STORAGE ATMOSPHERE

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(With Plate VIII.)

CONTENTS.

Introduction.

Some years ago the author, using acetaldehyde accumulation as an index of zymasis, showed that although Newton Wonder apples may contain traces of acetaldehyde and ethyl alcohol, they did not undergo progressive zymasis when stored at 1° C. from October to July. At 15° C. and 22° C. fungal rot commenced after February, but until that month no zymasis had taken place (17). It was concluded that zymasis does not occur in apples stored in CO_2-free air so long as they remain healthy—a vague term the meaning of which will be discussed later. This was the starting-point of the work described in this series of papers on the connection between zymasis and the physiological diseases of storage (18). The aim was to determine whether products of zymasis are to be found in apples

[1] The title of this series of papers is now altered to a more general form.

suffering visibly from these diseases, and if found, whether they started to accumulate before the incidence of the unhealthy state, or whether zymasis occurs only as a secondary phenomenon. It was thought that experiments on these lines might throw light on the causes of these diseases, and might also afford chemical means of diagnosing them. Here will be considered only those diseases and injuries which occur in the presence of oxygen and the absence of accumulations of carbon dioxide in the storage atmosphere. Bruising injury may of course be incurred in any form of storage, and many fruits are liable to freezing injury at temperatures below 0° C. Less easy to account for are the tissue brownings seen in fruit stored in freely circulating air above 0° C., although the suggestion has been made that certain volatile products of metabolism—for example, acetaldehyde (13)—may be toxic to tissues, and the successful use of oiled paper in lessening losses of marketable apples during storage gave some support to this idea.

Several distinct diseases of air storage are generally recognised, and these have been named by terms describing the different incidence of injuries and the variations shown in their subsequent extension (see detailed account by Kidd and West (8)). In the present paper only a few of these diseases will be mentioned. There is low temperature "internal breakdown" which starts in the outer flesh tissue and then spreads and deepens[1] (see Plate VIII, figs. 4 *A*, *B*, *C*); and "deep or soft scald" which is incident in the epidermis, and then extends inwardly and laterally (see Plate VIII, fig. 3); and "superficial scald" which only affects the epidermal tissue.

In the introduction to Part I of this series of papers it was pointed out that such names are not wholly satisfactory as the same browning sequences may sometimes be brought about by quite different conditions. For example, it is impossible by eye to distinguish between deep scald incurred in air stores and either invasive alcohol poisoning, which sometimes follows anaerobic conditions, or invasive aldehyde poisoning which may be met with after storage in certain gas mixtures of carbon dioxide and oxygen (see (18)). Moreover freezing injuries or bruising injuries may sometimes resemble deep scald. In this paper deep scald, a name which has often been loosely used in the past (see for example (2), (7), (17)), will connote invasive injury occurring in the presence of oxygen (thus excluding invasive alcohol poisoning), quite independently of the presence of carbon dioxide in the air (thus excluding invasive aldehyde poisoning),

[1] There are at least two forms of internal breakdown (10). Only low temperature internal breakdown will be discussed in this paper.

at temperatures higher than 0° C. (thus excluding freezing injury), and known not to have been brought about by bruising.

The methods of analysis by which the results, which will now be presented, were obtained have been already fully described (17). The experimental work was, however, shortened in some cases by determining "total zymasis," *i.e.* the total quantity of substances volatile in steam which give acetic acid on oxidation with potassium dichromate and sulphuric acid. Since in every instance the amount of ethyl alcohol was found to be much greater than that of acetaldehyde, "total zymasis" can be taken to represent approximately the concentration of ethyl alcohol in the tissue at the time of the analysis. The concentrations of acetaldehyde and ethyl alcohol found in healthy apples stored in air are on the average of many experiments as follows: acetaldehyde 0·0005 gm. per 100 gm. fresh weight of tissue, ethyl alcohol 0·006 gm. per 100 gm. fresh weight of tissue.

Section I. Zymasis and the physiological diseases incurred in CO_2-free air stores above 0° C.

In Table I are recorded a selection from a miscellany of results obtained, during the last 5 years, by analysing apples suffering from physiological diseases known to have been incurred in CO_2-free air stores above 0° C.

In drawing some of the following conclusions from these results, and also later in the paper, comparisons will be made with the findings of Harley and Fisher in their recent work on Bartlett pears (5).

1. Zymasis had not occurred either in the sound apples taken from samples showing low temperature internal breakdown, browning of the core, deep scald, superficial scald, or in the sound parts of individual apples suffering from these diseases. When apples and tissues are described as "apparently sound" it is probable that although injured cells were present these had not disorganised to the point of browning. Hence the slight zymasis shown by the "apparently sound" Cleopatras and Jonathans may be due to the incomplete separation of the injured samples of apples or parts of an apple from the uninjured fruit or tissue. If cell disorganisation can proceed for a period without tissue browning, then the observation of Harley and Fisher that acetaldehyde may accumulate in ripening pears before discoloration is seen, does not necessarily mean that zymasis was occurring in healthy cells: this important point will be discussed later.

Table I.

Zymasis in relation to diseases in CO_2-free air stores at temperatures greater than 0° C.

	Variety of apple used	Description of state of apple or tissue used	Acetaldehyde (%)	Total products of zymasis (acetaldehyde and ethyl alcohol) (%)
Exp. 1.*	Bramley's Seedling	Sound	0·0005	0·006
		Whole apples showing slight low temp. internal breakdown	0·002	—
		Whole apples showing severe low temp. internal breakdown	0·008	0·056
		Apparently sound tissue of apples showing low temp. internal breakdown	0·0006	0·001
Exp. 2.†	Cleopatra (Australian)	Apparently sound	0·001	0·008
		Whole apples showing very severe browning of the core of recent occurrence	0·004	0·09
		Apparently sound tissue of apples showing browning of the core	0·0008	0·01
Exp. 3.‡	Jonathan (New Zealand)	Whole apples suffering from severe deep scald	0·006	0·15
		Apparently sound tissue of apples suffering from deep scald	0·002	0·015
Exp. 4.§	Newton Wonder (home grown)	Sound	0·0005	0·006
		Whole apples suffering from superficial scald	0·0005	0·006

* These analyses were made in June 1924 on apples stored from the previous October at 1° C. in freely circulating air at the Low Temperature Research Station, Cambridge. [For appearance see Plate VIII, figs. 4 *A*, *B*, *C*.]

† Core browning was seen in these apples when they were unloaded from a ship in June 1927. Dr West sent them to the author, at Newcastle, by rail. According to the records kept on ship the apples had been stored in freely circulating air. In many respects the appearance of these apples in section was similar to that shown in severe "brown heart." [See Plate XX, fig. 2 (*b*) in earlier paper (17).]

‡ Deep scald was seen in these apples when they were unloaded from a ship in June 1927. Dr West sent them to the author, at Newcastle, by rail. According to the records kept on ship the apples had been stored in freely circulating air. The appearance typical of that shown by these apples in section is illustrated in Plate VIII, fig. 3.

§ These analyses were made in June 1924 on apples stored from the previous October at 1° C. in freely circulating air at the Low Temperature Research Station, Cambridge.

2. When the flesh tissue of an air-stored apple is suffering from any of the physiological diseases mentioned above, analysis shows that ethyl alcohol and acetaldehyde have accumulated in the diseased parts independently of the incidence of the injury. Harley and Fisher have shown

that zymasis accompanies breakdown and scald in pears. Moreover, estimations of acetaldehyde accumulation in Exp. 1 suggest that the amount of zymasis increases with increasing severity of breakdown: this is in accord with the positive correlation found by Harley and Fisher between the severity of scald and breakdown and the concentration of acetaldehyde in the injured pear tissue. It is probable that when acetaldehyde is found to accumulate in these fruits there will be an even greater increase in total zymasis (see (12) and (17)).

3. The concentrations of acetaldehyde or ethyl alcohol found in the injured tissues never reached the levels at which either aldehyde or alcohol poisoning sets in (see (18)). Caution is necessary in drawing conclusions from the results of Exps. 2 and 3, as the analyses were made many days after the occurrence of injuries and some loss of products of zymasis, particularly acetaldehyde, through escape or in some other manner, may have taken place. Harley and Fisher, in some of their experiments on Bartlett pears, found much higher concentrations of acetaldehyde: for example, pears removed from low temperature stores and ripened for 7 days at 15–18° C., and showing then 100 per cent. surface scalding and some core discoloration, returned on analysis 0·034 per cent. acetaldehyde—a concentration which, when found in apples after CO_2-zymasis, is often accompanied by invasive and internal aldehyde poisoning (18). Harley and Fisher definitely suggest that acetaldehyde is a "possible causative agent in the production of scald and breakdown in Bartlett pears." It is not thought, however, that the amounts of zymasis recorded in Table I justify the drawing of a similar conclusion for internal breakdown and deep scald in apples. The positive correlations between zymasis and disease in apples would hold equally well even if zymasis did not occur until after injury has commenced, and then increased progressively as injury became more profound. This possibility will be explored in the next section and it will be considered there whether, even in pears, in spite of the higher concentrations of acetaldehyde, zymasis may not be a secondary phenomenon following injury and not causal of it, *i.e.* whether in apples and pears zymasis only occurs in cells which for quite other reasons must be described as unhealthy.

Section II. Zymasis following freezing injury and mechanical injury and its significance in relation to Section I.

According to the thesis of Harley and Fisher zymasis occurs as a normal phase in the ripening of pears, and the acetaldehyde formed is responsible for the tissue injury which follows. They suggest that the

present writer's failure to detect acetaldehyde production in apples during air storage may be due to the slower ripening of this fruit. But it must be pointed out that sound apples nearly a year old, which had been yellow for many months—and therefore presumably could be described by the vague term ripe—have never returned on analysis appreciable quantities of ethyl alcohol or acetaldehyde. More probably a ripe pear is in a different physiological state from a ripe apple, and the presence of acetaldehyde in ripe pears may be a criterion of this state. Pears are certainly most highly esteemed by the consumer when they are so juicy that liquid flows out from cut surfaces in considerable quantities; apples do not pass through a similar phase that pleases the public. This difference is important, for it suggests that intercellular spaces in ripe pears are in part injected with liquid, whereas this is not necessarily the case in ripe apples. Further, it is well known that the ripe phase in pears is relatively brief, and is quickly succeeded by a phase of which the public disapproves and describes as overripe. In pears there is a rapid succession of unhealthy senescent states, the earlier of which is prized by the public; in apples there is no similar unhealthy state considered to be good eating.

It is not proposed to consider here the difficult question of what precisely is meant by the term "healthy cell," but it is submitted that, as a rule, such a cell will show medium permeability and so will not lose liquid by exosmosis of cell sap. It then follows that fruit tissue is not physiologically healthy, whatever the public may think about it, when irreversible increase in the permeability of the protoplasts of the constituent cells is brought about by toxic substances, normal ageing, or in any other way. Winkler, by finding a speedy decrease in the electrical resistance of apple tissue, subjected to the vapours of certain esters and aldehydes, has demonstrated such an increase previous to visible injury (19). Sometimes, as in ripening pears, intercellular spaces may be injected; in other cases, the rate of evaporation in the interior or reabsorption by other parts of the fruit may be sufficiently rapid to prevent injection. According to this view, apple or pear tissue may be unhealthy before discolorations are seen, although browning will occur later, if oxygen is present, when the components of the catechol-oxidase system come together through increase in the permeability of the plasmatic membranes which keep these components apart in healthy cells. It would then follow that whereas ripe pears are physiologically unhealthy and over-ripe pears more so, unripe pears and unripe and ripe apples are not necessarily unhealthy. It should be noted that none of the above state-

ments precludes a cell, still possessing medium permeability and not losing liquid water, from being unhealthy for quite other reasons. At the end of this section a chemical criterion of health in an apple or pear will be suggested.

It seemed probable that some light might be thrown on the relation between zymasis and injury by artificially injuring previously physiologically healthy fruit at an early stage in the storage season, and then investigating the form of autolytic metabolism which follows. Freezing injury was inflicted by placing the fruit in rooms either at − 5° C. or at − 10° C. for a period; the fruit became more or less hard according to the temperature used and the time of exposure; on thawing, the injured parts turned brown and exosmosis of liquid cell sap occurred. A photograph of a Jonathan apple after freezing injury is shown in Plate VIII, fig. 2. Mechanical injury was inflicted by beating the fruit with a porcelain pestle, care being taken not to break the skin which is known to be effective, when intact, as a barrier against micro-organisms. A photograph of half of an apple bruised in this way and then left for 3 days in air is shown in Plate VIII, fig. 1. The analytical results are recorded in Table II.

The following conclusions are drawn[1]:

1. Zymasis occurs during the thawing of artificially frozen, but previously healthy, apples and pears, provided the temperature used to freeze the fruit is not too low. The zymase system may be inactivated at temperatures less than − 10° C. (see (17)), possibly owing to the complete disorganisation of cells. This would explain why in Exp. 1 much less zymasis took place during 3 days at ordinary temperatures after 7 days at − 10° C., than after 7 days at − 5° C.

2. As a result of bruising injury, previously healthy apples and pears undergo zymasis.

Clearly, by freezing and by mechanical injury, similar metabolic states have been produced in immature fruits to those found in pears during late senescence before and during browning, and in apples in late senescence during browning. Probably zymasis may follow many other forms of tissue injury, as a secondary phenomenon, provided the zymase system has not been inactivated. Two possible interpretations of this change of metabolism will now be considered.

[1] As the purpose of these experiments was to ascertain whether zymasis followed artificial injury to healthy pears and apples, it is not proposed to consider here other matters, such as the differences in resistance to freezing injury offered by pears and apples, suggested by the results in Table II.

Table II.

Zymasis brought about by artificially injuring healthy fruit at an early stage of storage.

	Variety of fruit used	Experimental treatment of fruit*	State of fruit when analysed	Total zymasis (%)
Exp. 1.	Newton Wonder apples	2 months at 1° C. 3 days at ordinary temperatures	Sound, green	0·005
		2 months at 1° C. 7 days at −5° C. 3 days at ordinary temperatures	Flesh tissue severely injured, but not completely brown	0·08
		2 months at 1° C. 7 days at −10° C. 3 days at ordinary temperatures	Flesh tissue completely brown	0·01
Exp. 2.	Conference pears	2 months at 1° C. 3 days at ordinary temperatures	Sound, green-yellow	0·01
		2 months at 1° C. 7 days at −5° C. 3 days at ordinary temperatures	Flesh tissue apparently sound except for a few brown patches near the surface	0·025
		2 months at 1° C. 7 days at −10° C. 3 days at ordinary temperatures	Flesh tissue severely injured but not completely brown	0·04
Exp. 3.	Newton Wonder apples	2½ months at 1° C. 3 days at ordinary temperatures	Sound, yellow-green	0·006
		2½ months at 1° C. Bruising injury inflicted 3 days at ordinary temperatures	Cortical flesh tissue completely brown	0·1
Exp. 4.	Conference pears	2½ months at 1° C. 3 days at ordinary temperatures	Sound, yellow	0·015
		2½ months at 1° C. Bruising injury inflicted 3 days at ordinary temperatures	Cortical flesh tissue completely brown	0·1

* The fruit was picked in early October 1929, and stored in air in a room kept at 1° C. at the Low Temperature Research Station, Cambridge. It was either sent direct from this room, or after a period at still lower temperatures, by parcel post to Newcastle-upon-Tyne where it was analysed. The time spent in the post is counted as part of the exposure to air at ordinary temperatures, which, of course, fluctuated considerably.

Müller-Thurgau and Osterwalder (12) attributed the formation of ethyl alcohol[1] in ripening pears to a change of metabolism to an anaerobic type imposed by the blocking of intercellular spaces with liquid cell sap. They implied that an alteration in physiological state, initiated in an unknown manner, leads to an increase in permeability of the protoplasts and so to the partial or complete injection of the air spaces. Gaseous exchange may now be impeded, and the unhealthy tissue in the interior of a bulky fruit, such as a pear, may well become surrounded by an atmosphere poorer in oxygen and richer in carbon dioxide. Conceivably either CO_2-zymasis, or some form of anaerobic zymasis may follow as a secondary phenomenon. Whether this interpretation can hold for the cases of zymasis described in this paper, and in that of Harley and Fisher, must depend upon the results of analysis of the gas mixtures in the interior of fruits whilst injury is proceeding, and on a closer knowledge of the metabolism of cells in different senescent states in such gas mixtures. That conditions around diseased or injured tissue are not anaerobic is evident from the browning which usually follows. A simple experiment, however, showed that the catechol-oxidase is active in the presence of very little oxygen, for on passing a gas mixture of 99 per cent. nitrogen and 1 per cent. oxygen through amyl acetate and then over an apple a brown ring formed, after 24 hours, around each lentical. Now, since it has earlier been proved that zymasis occurs in apples in a gas mixture of this composition (17), it follows that browning and zymasis of an anaerobic type can proceed simultaneously in the same tissue. Indeed, whilst browning is taking place the oxygen shortage will become more extreme, for it is known that this process is accompanied by vigorous oxygen uptake (16). Results of gas analyses, of the internal atmosphere of Bartlett pears undergoing breakdown, reported in a recent paper by Harley (6), make it more probable that CO_2-zymasis is responsible for the accumulation in the fruit of ethyl alcohol and acetaldehyde. In no case was the oxygen concentration less than 5 per cent., so it is unlikely that any form of anaerobic zymasis was occurring; but occasionally, when values of carbon dioxide greater than 30 per cent. associated with oxygen concentrations less than 10 per cent. were found, it is quite likely that CO_2-zymasis would be induced.

It is open to question whether restricted gaseous exchange, following

[1] These authors also noticed the progressive accumulation of acetaldehyde in pears which were developing towards the "sleepy stage." They suggested that it was produced in the core tissue by the oxidation of the alcohol diffusing from the cortex. The present writer does not agree with this suggestion, but a discussion is out of place here.

injection of air spaces is invariably the cause of zymasis during injury or disease. Harley reports several cases in which acetaldehyde accumulated in browning pears when the internal concentration of carbon dioxide was less than 10 per cent., and the oxygen concentration greater than 15 per cent.—known to be non-lethal conditions for whole fruits, since gas storage is successful in such gas mixtures (10). Moreover, the present writer has found that loosely packed apple tissue, which had been previously pounded in a mortar, undergoes zymasis even in pure oxygen. These facts suggest that zymasis may sometimes occur as a phenomenon of cell disorganisation, independently of the composition of the gaseous environment. If this is the case, an alternative interpretation of the initiation of zymasis must be sought. Recent work on the respiration of sound apples and other plant tissues clearly shows that this complex process depends upon the co-ordinated activity of several enzyme systems in the living protoplasm (1, 4). Briefly, carbohydrates are believed to undergo zymasic cleavage to produce intermediate products which may be oxidised to carbon dioxide and water, or which may be changed back to the carbohydrate form by oxidative anabolism. It is suggested that the zymase component of the delicate cell machinery in control of these ordered sequences is less readily injured and inactivated than some one or more of the other components, and, therefore, when disorganisation sets in, from whatever cause, ethyl alcohol and acetaldehyde will accumulate, provided the zymase system itself is not inactivated, because the intermediate products are not removed at the rates which are associated with the usual respiratory metabolism. It is firmly established that the zymase system, acting by itself, can produce ethyl alcohol and acetaldehyde in the presence of oxygen (12). That a similar phenomenon may occur in slowly disorganising animal tissues is indicated by the detection of the accumulation of lactic acid in meat stored below 0° C. (15); in this case, however, a correlation between glycolysis and desiccation is suggested.

It will be seen that, on either of the two interpretations which have just been put forward, zymasis, in association with injuries and diseases recorded in this paper, or in the work of Müller-Thurgau and Osterwalder, Harley and Fisher, and Harley, must be considered as a secondary phenomenon, following a change of state of unknown origin in the tissue. It is not considered that Harley and Fisher, and later Harley have established that acetaldehyde is causal of breakdown and scald in pears. That this substance when formed may aggravate diseases or injuries already in progress is, however, quite probable.

Finally, it follows, from the line of argument developed in this section, that the accumulation of ethyl alcohol and acetaldehyde in pears and apples stored in air may be taken as a chemical criterion that the fruits are unhealthy. This is a promising approach to the problem of physiological state, as it directs attention to the fundamental process of plant respiration. In the animal world it is already known that, in certain diseases, products of incomplete oxidation accumulate in tissues and are eliminated from the body. It may be possible to compare later the functional disorders discussed in this paper with certain aspects of such animal diseases.

Section III. The relation between concentrations of ethyl alcohol and acetaldehyde in apples and the diagnosis of injuries and physiological diseases.

The following general conclusions are drawn, from the results recorded in Part I of this series of papers (18), and in Tables I and II of this paper, as to the use of determining the concentration of products of zymasis in apples suffering from disease or injury.

(i) Since there is no necessary connection between superficial scald and general zymasis in the flesh tissue of the affected apples (see Section I and Table I) it follows that, in the present state of our knowledge, estimations of ethyl alcohol and acetaldehyde will not throw light on this disease. Possibly the epidermal cells of apples undergo zymasis after they are injured, but there is as yet no evidence of this.

(ii) All the diseases and injuries so far encountered in air storage can be distinguished by chemical analysis from invasive alcohol poisoning, and from internal and invasive aldehyde poisoning (see Part I (18)), which are respectively incurred under anaerobic conditions and in certain concentrations of carbon dioxide and oxygen. We notice that the healthy flesh tissue of apples, which have suffered disease or injury in air stores, do not return abnormal concentrations of ethyl alcohol or acetaldehyde, whereas accumulations of one or both of these substances are found in the healthy flesh tissue of apples damaged under anaerobic conditions or under certain conjunctions of carbon dioxide and oxygen. Then, although ethyl alcohol and acetaldehyde are found in the diseased or injured parts of apples which have been stored in air, the concentrations of these substances are always different from those found in apples after anaerobic zymasis or CO_2-zymasis. Of particular importance is the fact that deep scald which occurs in air stores can usually be distinguished by chemical analysis from invasive alcohol poisoning, and from invasive

aldehyde poisoning, whereas from appearance alone no such distinction can be made. All these facts are brought out in Table III, and a comparison should be made between Plate XX, figs. 1 (*a*) and 2 (*b*), at the end of Paper I of this series, and Plate VIII, fig. 3, at the end of the present paper.

Table III.

Concentrations of ethyl alcohol and acetaldehyde found in apples after invasive injuries.

		Healthy flesh tissue of diseased apples		Diseased tissue		
Name of disease	Storage conditions under which disease may be encountered	Ethyl alcohol (%)	Acetaldehyde (%)	Ethyl alcohol (%)	Acetaldehyde (%)	Remarks
Deep or soft scald	Air storage. Especially prevalent at low temperatures, which are, however, greater than 0° C.	0·015	0·002	0·15	0·006	Maximum concentrations found as yet in any apple suffering from this disease
Invasive alcohol poisoning	Anaerobic conditions followed by exposure to air	0·35	0·004	0·35	0·004	Minimum concentrations as yet found in a single apple when disease was first noticed
Invasive aldehyde poisoning	High concentration of carbon dioxide in the presence of oxygen	0·07	0·03	0·07	0·03	Minimum concentration as yet found in a single apple when disease was first noticed

It is thought that results of this kind may be of importance in checking records of the composition of gas atmospheres in industrial stores or in holds of ships. For example, the experience of the author supports the contention of those responsible for aerating the ship's hold carrying the "New Zealand Jonathans" which were suffering from invasive browning when unloaded in this country (see Section I, Table I, Exp. 3); for, at no time during the voyage could there have been any considerable accumulation of carbon dioxide or conditions which were virtually anaerobic, otherwise zymasic products would have accumulated to a greater extent in the flesh tissue of the apples.

(iii) The results given in Tables I and II of this paper show that as yet deep scald, low temperature internal breakdown, frost injury and mechanical injury cannot be distinguished from one another by estimating the ethyl alcohol and acetaldehyde concentrations in diseased or injured apples.

Summary.

1. Ethyl alcohol and acetaldehyde do not accumulate in apples or pears stored in air so long as the fruit remains physiologically healthy—a term which is discussed in the text.

2. (*a*) Apples stored in air are liable, especially at low temperatures, which are, however, higher than 0° C., to suffer from "low temperature internal breakdown" and from "soft or deep scald." After the incidence of these diseases and as they become more profound, ethyl alcohol and acetaldehyde progressively accumulate in the unhealthy tissues. There is no evidence that the formation of these substances precedes the incidence of the disease.

(*b*) The view is put forward that for some biophysical or biochemical reason, not as yet known, cells become unhealthy and zymasis follows subsequently as a secondary phenomenon. Supporting this view is the fact that healthy apples at an early stage in their storage experience undergo zymasis after suffering artificial injury by freezing or bruising. It is suggested that after many types of tissue injury zymasis may follow. This chain of reasoning subordinates zymasis in air-stored apples to a pre-existing state of incipient disorganisation which may be brought about in many different ways.

(*c*) Diseases described in this paper are, therefore, of quite a different class from those described in Part I of this series, and chemical analysis will frequently distinguish internal breakdown and soft or deep scald from invasive aldehyde and invasive alcohol poisoning. Particularly important are the differences between deep scald and the invasive poisonings and it is urged, therefore, that the term scald should only be used for invasive diseases which occur, independently of carbon dioxide accumulation, in air stores above 0° C.

(*d*) It is argued that the results obtained by other workers on diseases of pears in air stores can be interpreted in the same way, as in 1 and 2 (*a*) and (*b*) above, although here zymasic accumulations may aggravate the progressing disease.

3. Two interpretations of the cause of zymasis are considered:

(*a*) Following the incidence of a state of ill health, exosmosis of cell sap occurs. Intercellular spaces become injected with liquid which hinders gaseous exchange. In the cells in those localities that consequently become poor in oxygen or very rich in carbon dioxide, zymasis occurs as a secondary phenomenon.

(*b*) Recent gas analyses of the internal gas atmosphere of diseased

pears suggest that (*a*) is not a universal interpretation. It is therefore suggested that, during disorganisation of the cells of pears and apples, the intricate co-ordination of enzymes in the respiratory centres of the protoplasm breaks down; then, so long as the zymase component remains active, carbohydrate cleavage proceeds in part, at least, all the way to ethyl alcohol and acetaldehyde.

4. The disease known as superficial scald is not necessarily preceded by zymasis in the whole apple which is suffering from it.

ACKNOWLEDGMENTS.

I wish to record my gratitude to Dr Franklin Kidd and Dr Cyril West of the Low Temperature Research Station, Cambridge, for the assistance they have given in organising this work, for reading and criticising the manuscript of this paper, and for permitting me to use several of their photographs.

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EXPLANATION OF PLATE VIII

Fig. 1. Bruising injury produced by beating a Newton Wonder apple with a porcelain pestle and then leaving for 3 days in air. (See Section II.)

Fig. 2. Jonathan apple suffering from freezing injury following exposure to temperatures less than 0° C. (See Section II.)

Fig. 3. Jonathan apple suffering from deep scald incurred at temperatures greater than 0° C. in CO_2-free air. Injury was incident in epidermal cells, progressed inwardly, and spread laterally. (See Introduction and Sections I and III; also compare with Plate XX, figs. 1 (*a*) and 2 (*b*) at the end of Part I of this series.)

Figs. 4 *A*, *B*, *C*. Incidence in the interior and spread of low temperature internal breakdown in a Bramley's Seedling apple. (See Introduction and Section I.)

(*Received May 9th*, 1930.)

Fig. 1.

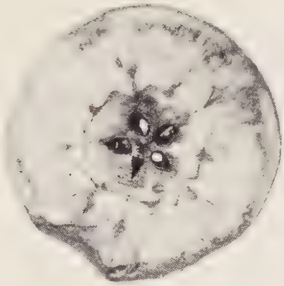

Fig. 2.

Fig. 3.

Fig. 4 *A*.

Fig. 4 *B*.

Fig. 4 *C*.

THOMAS.—The Production of Ethyl Alcohol and Acetaldehyde by Fruits in Relation to the Injuries occurring in Storage (pp. 60–74).

FURTHER RESULTS OF AN INVESTIGATION INTO THE RESISTANCE OF BASKET WILLOWS TO BUTTON GALL FORMATION

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(With Plates IX and X.)

CONTENTS.

I. Introduction.

This line of research is the natural outcome of an investigation into the bionomics of the button top midge (*Rhabdophaga heterobia* H.Lw.). A popular account of the life history and damage caused to the willow industry by this insect has already been published (Barnes, 1929) and control measures were then discussed. Subsequently, trials were set up to discover whether all the varieties and species of *Salix* used in commercial growing are equally liable to attack. A preliminary paper explaining the methods and results of the first year's trials, together with field observations, has recently appeared (Barnes, 1930). These results show clearly that all species of *Salix* are not equally susceptible to attack, and that a hybrid variety called Harrison (*viminalis* × *purpurea*) remained totally immune after three successive attacks by the midge in question.

II. Rothamsted trials, 1930.

(*a*) *Harrison immunity trial.*

In 1930 the variety Harrison was subjected to a more rigorous test of immunity. The method adopted was to grow plants in pots without the presence of any susceptible variety. Female midges were then intro-

duced into the cages at intervals throughout the season. The result was that, although eggs were occasionally laid on the plants, most of the midges did not oviposit. The eggs that were laid developed normally and the larvae hatched. They subsequently died, since apparently they were unable to feed, and the plant did not react or form button galls. No side shoots were produced and no shoots died as a consequence of the eggs being deposited.

(*b*) *Preference trials.*

Trial beds of different varieties were set up as in 1929 with the exception that twice as many plants were planted in each cage and that twenty-five, instead of sixteen to twenty, female midges were placed in each cage at the beginning of the experiment. In one bed there were three varieties of *S. triandra* (Whissender, Grisette and Sarda), and one variety of *S. purpurea* (Welch); in the second bed there was one variety of *S. triandra* (Black Maul), one variety of *S. viminalis* (Long Skin), and two hybrids, *S. viminalis* × *purpurea* (Pyramidalis and Mawdesley); in the third bed there were two varieties of *S. triandra* (Pomeranian and Stone Rod) and two varieties of *S. purpurea* (Dicky Meadow and Brittany Green); and in the fourth bed there were four varieties of *S. triandra* (Mottled Spaniard, Long Bud, Dark French, and Black Maul). (See Table I.)

Table I.

Plan of the 1930 *Rothamsted preference trials. Arrangement of the different varieties.*

Two sets of each variety were planted in each position.

Bed 3		Bed 1	
Pomeranian	Stone Rod	Whissender	Grisette
Dicky Meadow	Brittany Green	Sarda	Welch
Brittany Green	Pomeranian	Welch	Whissender
Stone Rod	Dicky Meadow	Grisette	Sarda
Pomeranian	Brittany Green	Whissender	Welch
Dicky Meadow	Stone Rod	Sarda	Grisette
Brittany Green	Pomeranian	Welch	Whissender
Stone Rod	Brittany Green	Grisette	Welch
Pomeranian	Dicky Meadow	Whissender	Sarda

Bed 4		Bed 2	
Mottled Spaniard	Black Maul	Long Skin	Black Maul
Dark French	Long Bud	Pyramidalis	Mawdesley
Long Bud	Mottled Spaniard	Mawdesley	Long Skin
Black Maul	Dark French	Black Maul	Pyramidalis
Mottled Spaniard	Long Bud	Long Skin	Mawdesley
Dark French	Black Maul	Pyramidalis	Black Maul
Long Bud	Mottled Spaniard	Mawdesley	Long Skin
Black Maul	Long Bud	Black Maul	Mawdesley
Mottled Spaniard	Dark French	Long Skin	Pyramidalis

The type of cage used was the same as in the 1929 trials at Rothamsted and is illustrated in Plate IX, fig. 1. Plate IX, fig. 2, shows the inside of the cage in which three susceptible varieties and one immune variety were growing in the 1929 experimental bed 1. Plate X shows three typical rods of each of the four varieties: *A*. Newkind; *B*. Harrison; *C*. Black Maul; *D*. Champion from the same bed.

Twice as many plants were put in each cage in 1930 as in 1929 (eight to ten plants instead of four to six); this ensured that there would be a greater number of shoots available to the first midges (parents) when introduced into the cages. These measures allowed for the statistical examination of the results, if necessary, in relation to differential attack.

Table II.

The result of the 1930 *Rothamsted preference trials.*

Variety and species	No. of shoots available to: Parent midges	F_1 midges	No. of button galls produced by: Parent midges	F_1 midges	No. of shoots killed but without gall formation by: Parent midges	F_1 midges	Percentage attack by: Parent midges	F_1 midges
Bed 1								
Whissender, T.	27	41	17	34	7	7	89	100
Sarda, T.	19	53	9	46	6	6	79*	98*
Welch, P.	32	23	0	0	0	0	0	0
Grisette, T.	22	26	17	23	3	3	91	100*
Bed 2								
Long Skin, V.	35	31	0	0	0	0	0	0
Pyramidalis, V. × P.	24	14	0	0	0	0	0	0
Mawdesley, V. × P.	40	16	0	0	0	0	0	0
Black Maul, T.	18	16	13	13	5	3	100	100*
Bed 3								
Pomeranian, T.	27	3	15	3	6	0	77	100*
Dicky Meadow, P.	22	16	0	0	0	0	0	0
Brittany Green, P.	32	17	0	0	0	0	0	0
Stone Rod, T.	22	0	17	0	1	0	82	0†
Bed 4								
Mottled Spaniard, T.	30	69	20	64	8	5	93	100*
Long Bud, T.	23	78	15	74	5	4	87	100*
Dark French, T.	41	49	16	39	13	10	71	100*
Black Maul, T.	29	35	19	33	8	2	93*	100*

T. = *S. triandra*. P. = *S. purpurea*. V. = *S. viminalis*.

V. × P. = Hybrid *S. viminalis* × *purpurea*.

* Lateral bud attack in addition to terminal or button gall formation.

† Lateral bud attack when no terminal shoots were available for button formation.

Some plants were taken out and others thinned out after parent midge attack, this accounts for fewer shoots being available to the F_1 midges than to the parents.

Twenty-five female midges, or parents, were placed in each cage at the beginning of the experiment to ensure a large percentage of damage,

and also, if possible, to secure a 100 per cent. attack of available shoots of the susceptible varieties by the second or F_1 flight of midges.

The parent midges were introduced through the sleeves of the cages on May 25th to 28th. By the latter date twenty-five impregnated females had been placed in each of the four cages. Observations were carried out at very frequent intervals during these 4 days, and oviposition was seen to be taking place on all the varieties of *S. triandra*, but on none of the other varieties. On June 6th the first signs of button gall formation were noticed, and on June 23rd to 25th the button galls of the first attack were counted. On July 2nd the F_1 midges were flying about in the cages for the first time. On July 29th–31st the button galls of the second attack were counted, and it was noticed that some of the plants had not freely developed side-shoots and that some plants of Stone Rod variety had died. Further, a considerable number of lateral buds on several varieties had been galled.

The results of the first two attacks by the parent and F_1 midges can be seen in Table II. Here the varieties and species of willow are set out, together with the number of terminal shoots available for attack, the number of button galls formed, and the number of shoots killed without gall formation. In cases where sufficient terminal shoots are not available for oviposition by the midges the lateral buds are also galled. In the table this lateral bud attack is denoted by an asterisk.

(c) *Immunity trials.*

Immediately after the button galls of the first attack had been counted in beds 1 and 3, two plants each of Welch and Brittany Green varieties were isolated, and covered with small muslin cages, inside the large cages. Female midges were then introduced within the small cages, and it will be obvious that they had to lay on the Welch and Brittany Green or fail to oviposit. These small cages were taken off before the flight of the F_1 midges, so that there would be further chances of their being attacked. No galls or side-branching occurred on either variety.

After the second attack in the large cages had been counted, the susceptible varieties in beds 1 and 2 were removed, and pots containing galls with larvae and pupae were placed in the cages. Thus, when the midges emerged the remaining immune varieties were subjected to a further attack. The number of midges estimated to be present in this attack (during August) was between 500 and 1000 of both sexes. In this way Welch, Long Skin, Pyramidalis and Mawdesley were given an immunity trial. In bed 4, where there were originally four susceptible

varieties, *S. alba* var. *vitellina* was planted and subjected to an immunity trial. None of these varieties showed either button gall formation or side-branching, neither was normal oviposition observed on them.

Besides these trials in the beds, small cages were set up over plants in pots in the case of the following varieties: Dicky Meadow and Pyramidalis. Into these cages midges were introduced at various times during the season. A few eggs were laid on Pyramidalis but none on Dicky Meadow. The larvae hatched but perished, neither galls nor side-branching occurred in either variety.

III. SUSCEPTIBILITY OF *SALIX TRIANDRA*.

Six varieties of *S. triandra*, viz. Champion, Black Maul, Newkind, Light French, Stone Rod and Pomeranian, were tested in 1929, and it was found that although all were highly susceptible, some, *e.g.* Light French, did not always form button galls when attacked, the shoots dying instead. In these cases side branching occurred just as in the cases where galls were formed. Nevertheless such varieties would be useful in that they prevent the midges from multiplying as the larvae cannot survive without the formation of galls.

In 1930 six additional varieties were tested, viz. Whissender, Sarda, Grisette, Mottled Spaniard, Long Bud and Dark French. All these varieties were heavily attacked. Stone Rod, Black Maul and Pomeranian were also tested again, and some of the first-named variety apparently were killed by the first attack, and had not strength enough to produce side-shoots.

It will be seen, therefore, that all twelve varieties of *S. triandra* are very susceptible to attack. In addition Champion, Newkind, Light French, Sarda, Grisette, Black Maul, Pomeranian, Stone Rod, Long Bud, Mottled Spaniard and Dark French suffered from lateral bud attacks. Whissender, which was the remaining variety used, suffered no lateral bud attack.

IV. IMMUNITY OF *SALIX VIMINALIS*, *S. PURPUREA* AND *S. ALBA*.

One variety of *S. viminalis*, viz. Long Skin, was used in the preference and immunity trials in 1930 and no button galls or side-shoots, resulting from attack by the midge, occurred. No eggs were found on the plants and no oviposition was observed.

Three varieties of *S. purpurea*, namely Welch, Brittany Green and Dicky Meadow, were subjected to similar trials. The result was the same, no galls, no dying of terminal shoots, no eggs laid and no oviposition

observed. It is possible that the surface of the leaves may be too smooth for the midges.

One variety of *S. alba* var. *vitellina*, viz. Golden, was given an immunity trial in 1930. No midges were seen to deposit eggs, with the exception of one female which became fortuitously glued on to a bud and oviposition apparently became obligatory. These eggs developed but the larvae died. Neither galls nor dying of terminal shoots occurred.

V. Immunity of hybrids *S. viminalis* × *purpurea*.

Three hybrids, Harrison, Mawdesley and Pyramidalis, were used in the experiments in 1929 and 1930, each receiving preference and immunity trials. Although eggs were laid occasionally, and in small numbers, on Harrison and Pyramidalis in the immunity trials and the larvae hatched, no galls or dying of terminal shoots were observed on any of the three hybrids.

VI. Discussion.

From the experiments described it will be seen that all the varieties of *S. triandra* used were very susceptible, but that all varieties of *S. viminalis*, *S. purpurea*, *S. alba* and the hybrid *viminalis* × *purpurea* were totally immune. These results are confirmed by field observations. Unfortunately *S. triandra* is the species grown most extensively for the basket industry, while the other species and their varieties are not so much used except certain varieties of *S. viminalis*, *e.g.* Long Skin, some varieties of *S. purpurea*, used for fancy basket work, and *S. alba* var. *vitellina*. While *Rhabdophaga heterobia* is by no means the only insect pest of basket willows it is a species that cannot be controlled by spraying. It will be obvious, therefore, that the discovery of resistant varieties of willows is of particular significance in this case. On the other hand, another species of gall midge, viz. *Rhabdophaga terminalis* H.Lw., is so far only known to attack certain varieties of willows immune to infestation by *R. heterobia*. Of the two species of insect the last mentioned is the most widespread pest, while the former is sometimes very injurious locally. Hutchinson and Kearns (1930) have stated that, among Chrysomelid beetles, *Galerucella lineola* appears to be confined to *S. triandra* varieties, but *Phyllodecta vitellinae* will attack many species of willows, particularly *S. purpurea* and its varieties and *S. alba* var. *vitellina*, while *S. triandra* is never attacked. The whole subject, therefore, of the control of willow-infesting insects is a complicated one. Aphides and sawflies have also to be included among their enemies.

Mr H. C. F. Newton of Rothamsted is now investigating the chemotropic responses of the button top midge in an attempt to find out what is the cause of the immunity exhibited by certain species of *Salix*.

It is suggested that, if possible, hybridisation between *S. triandra* and one of the species of *Salix* immune to the button top midge might be attempted. For example, observations on the trial plots of the Long Ashton Research Station revealed that Black Top, a hybrid *S. viminalis* × *triandra*, was free from attack during 1927 and 1928. If it were found possible to do this, the resultant cross might carry the factor or factors causing immunity and the characters, *e.g.* for whiteness, so much desired by the growers.

Finally, in dealing with host plant preference of willow gall midges, three aspects warrant special attention. Firstly, in view of the high specialisation of the gall midges in general, the possibility of biological races must not be ignored. All the midges used in the trials dealt with in this paper were collected from one very restricted locality in Leicestershire. It is quite possible that a race may arise (or has arisen) which will thrive on the varieties[1] which have so far proved to be immune. Secondly, in the literature on plant galls, statements such as the following are frequent: *R. heterobia* recorded on *Salix aurita, amygdalina, caprea, fragilis, purpurea, repens, triandra*. It is extremely doubtful if such statements can be substantiated. Frankly, it is best to ignore them until further proof can be given, because (1) the identification of the species of *Salix* and of the midge may be at fault, (2) the identification of the midge solely by its gall, at any rate among the genus *Rhabdophaga*, which is almost entirely confined to *Salix* spp., is untrustworthy, and (3) such critical experiments as have been made show that species of gall midges are restricted very definitely to particular species of host plant. Thirdly, at present only commercial varieties of *Salix*, and species of midges found in commercial willow beds, are being used by the writer in his experiments.

VII. SUMMARY.

1. Twelve commercial varieties of *S. triandra* have been proved, under experimental conditions, to be very susceptible to attack by the button top midge (*R. heterobia*).

2. Three varieties of *S. purpurea*, one variety of *S. viminalis*, three

[1] There is a midge at present unidentified whose larvae live in the terminal shoots of Long Skin or Skein (Dark and Green) and Continental, both varieties of *S. viminalis*, in Lancashire willow beds. No gall is formed but the terminal shoot dies. These larvae are very similar to those of the button top midge (*R. heterobia*).

hybrids of *S. viminalis* and *purpurea* and *S. alba* var. *vitellina* have proved to be totally immune. Notwithstanding eggs sometimes being laid and the larvae hatching, no galls or side-branching have occurred.

3. From field observations a closely allied gall midge, *R. terminalis*, is known to attack *S. alba* var. *vitellina*, but has not been known to attack any variety of *S. triandra*.

4. Reference has been made to a similar phenomenon with regard to attacks on willows by the Chrysomelid beetles; viz. to *Galerucella lineola* which appears to be confined to varieties of *S. triandra*; and to *Phyllodecta vitellinae* which is known to attack *S. alba* var. *vitellina* and *S. purpurea* and its varieties, while it is not known to attack any variety of *S. triandra*.

5. It is suggested that hybridisation of *S. triandra* × *S. purpurea* or × *S. viminalis* or × *S. alba* should be attempted. Mention is made of three aspects of host plant preference needing special attention in connection with willow gall midges.

In conclusion it is desired to express the indebtedness the writer owes to Mr Hutchinson, who was responsible for the choice of the varieties of willows to be tested in the various trial beds and the supply of sets. Dr Walton, of the Long Ashton Research Station and Mr Kearns of Bristol University have taken part in several very helpful conversations. Thanks are also due to Dr Imms for the generous aid he has given throughout the period of the investigations.

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EXPLANATION OF PLATES IX—X

PLATE IX.

Fig. 1. View of cage inside greenhouse.

Fig. 2. Plants growing inside the cage showing Harrison immune to attack.

PLATE X.

Three typical shoots from cage 1, 1929, showing *A*. Newkind, *B*. Harrison, *C*. Black Maul and *D*. Champion.

(*Received August* 30*th*, 1930.)

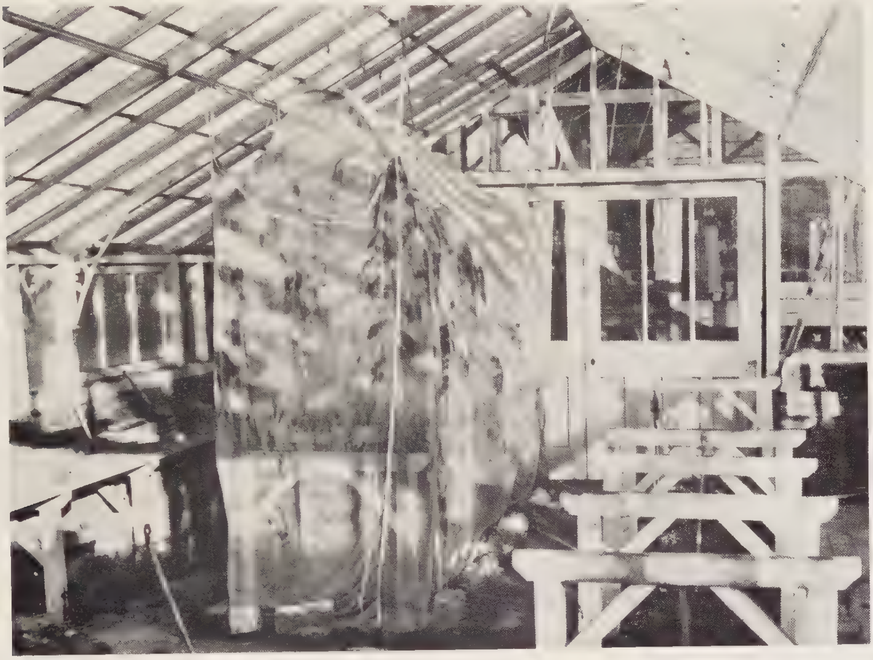

Fig. 1.

Fig. 2.

BARNES.—Further Results of an Investigation into the Resistance of Basket Willows to Button Gall Formation (pp. 75–82).

A.

B.

C.

D.

BARNES.—Further Results of an Investigation into the Resistance of Basket Willows to Button Gall Formation (pp. 75–82).

THE STEM AND BULB EELWORM, *TYLENCHUS DIPSACI* (KUHN) BASTIAN. A FURTHER CONTRIBUTION TO OUR KNOWLEDGE OF THE BIOLOGIC STRAINS OF THE NEMATODE

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Introduction.

The nematode, *Tylenchus dipsaci*, is of such importance from both the agricultural and horticultural viewpoint that no excuse need be made for again approaching the subject of the occurrence of biologic strains of the worm. The term biologic strain, as applied to *T. dipsaci*, indicates a strain of the nematode which, while morphologically indistinguishable from any other strain, is restricted for the purpose of feeding and reproducing to a specific host plant.

The problem which confronts one may briefly be expressed thus. Is each strain of such remote origin as to have acquired practically the significance of a species, in which case rigid attachment to the selected host plant might reasonably be expected? Alternatively, have strains but recently acquired the predilection for the plant in which they are found, when transfer from one host to another might well be feasible? The practical significance of these questions is clear. Let us assume for a moment that each strain is restricted to a specific host, or in a limited number of cases to two hosts. Then our present knowledge of the biology of the worm shows that nothing more than a simple rotation of crops, assisted in some cases by a starvation fallow, will effect a control to the extent of preventing damage to subsequent similar crops. On the other hand, if our assumption is unsound and it were found that a ready interchange of hosts is of common occurrence, the problem would assume a very different aspect.

The chief purpose of the present publication is to present experimental evidence concerning the behaviour of certain strains of the nematode when afforded opportunities of entering hosts other than those in which they originally occurred. In passing it may be suggested that morphologists in this country might profitably conduct a detailed com-

parison of specimens of the nematode from a range of host plants. The term morphologically indistinguishable, as opposed to morphologically identical, is used advisedly in the first paragraph; for, during a recent visit to the Continent, the writer was afforded evidence that strains from two particular plants could be separated with a considerable degree of accuracy on purely morphological grounds.

Historical.

In a previous publication (2) the writer discussed the biologic strain theory in some detail. Field observations in support of the theory were cited and various field and cylinder experiments described. These latter threw some light on the behaviour of the nematodes when given access to host plants other than those in which they had previously fed. The experiments were not extensive enough to form a basis for any final conclusions, the position at that time being best indicated by quoting paragraph 5 of the summary: "Examination of available data indicates that while biologic strains or races of the eelworm do exist, these strains show varying powers of adaptation to other host plants." This finding agrees in part with that of Steiner (4), who as a result of a careful study of available data concerning both *T. dipsaci* and other plant parasitic nematodes, concluded that the preference for a specific host became more and more definite with every succeeding generation on that host. Steiner, however, goes on to say that: "Forced by starvation, a nema population will attack a host which it will completely ignore if a more preferred one is present." With this the writer cannot entirely agree, for during the past five years a considerable addition has been made to the data available. As will be shown, the present series of experiments strongly suggest that Steiner's hypothesis is by no means generally applicable to *T. dipsaci*, or at least to the majority of those strains examined by the writer.

An account has recently been published (3) of the occurrence of the nematode in certain weeds, namely *Hypochaeris radicata*, and *Plantago* spp. in south-west England. Strains from these hosts have been given some prominence in the experiments to be described. This has been done for the purpose of determining the likelihood of the transference of attacks from weeds to cultivated plants and *vice versa*.

It was felt that the experiments would be of most value if restricted to a limited number of potential host plants of the nematode, for, as shown recently by Goodey (1), the full list of such plants is so large that no one series of experiments could possibly cover the whole field. Further,

a comparatively small number of strains of the nematode were employed, those known to have inhabited a single host for some years being given preference when possible. Each strain was invariably used for a number of experiments, but it must be understood that in every case a stock was kept breeding uninterruptedly in its own host plant during the whole period, portions being removed from time to time as required.

Experience gained in previous work indicated that a reasonably small number of repetitions of each experiment were necessary. In earlier experiments as many as fifteen separate containers were used each time. Invariably such uniformity of results was obtained, once the technique of successful inoculation with nematodes was mastered, that it was considered sufficient to limit the number to five containers, of which two were uninoculated controls.

The containers used for many of the experiments were zinc cylinders 2 feet in depth and 9 inches in diameter. These were open at each end and were sunk into the ground to within 4 inches of the top. Each was filled with nematode free soil prior to commencing an experiment. It was found that 12-inch earthenware pots could also be relied upon to give reliable results, particularly with bulbous plants. When pots were employed they were kept well apart, either on the dry concrete floor of a shed, or on the staging of a glasshouse. All watering was done with tap water in order to minimise the chances of accidental infection.

Mode of infection.

Prior to the commencement of any further host transference experiments, considerable attention was paid to a determination of the most simple and reliable method of releasing nematodes into the soil. For this purpose the nematodes were given access to healthy plants similar to those in which they had been breeding. Various methods were compared. The first consisted of cutting up infested plants, in either tap or distilled water, allowing time for the nematodes to escape into this and then watering the whole on to the desired material. Results obtained by these means proved to be quite irregular, for, in at least 25 per cent. of the cases, the nematodes failed to attack the plants provided. As an alternative, freshly sliced portions of infested material were incorporated directly with the soil around the plants in which it was desired to establish the nematodes. On some occasions the soil was watered immediately after the infested material had been added, and on others after intervals of 24, 48 and 96 hours. The results were even less uniform than before, for, in only about 60 per cent. were attacks established, and this

regardless of the times of watering. It was assumed that the rapid fermentation and decay of the cut portions of plant killed many of the nematodes before they were able to enter the soil. Also, by both this and the previous method, those nematodes which entered the soil unharmed were not always able to enter a fresh plant. In all probability the chief contributory cause to the failure of these methods lay in the suddenness with which the nematodes were removed from their feeding ground, many being in a physiological condition quite unsuited to journeying through the soil.

The method finally adopted for the present series of experiments consisted of cutting the infested material into fairly small pieces and allowing these to dry out completely before incorporating with the soil. The drying was effected over a period of from 5 to 8 days, being designed to allow the nematodes to enter gradually into a state of quiescence. Such material could then be used immediately or kept dry for a period of 4 years (this latter period is not presented as a maximum, but is merely the longest period over which material has been kept by the writer) and relied upon to set up an infestation in the specific host of the strain when required. Very few failures have been experienced with such material, and these were attributed to the too rapid drying, as a result usually of accidental exposure to direct sunlight.

In passing it may be mentioned that one other method of producing an infestation has been used with some success. This consists of placing active nematodes directly into fresh incisions in healthy plants. In, roughly, 70 per cent. of the cases successful infestations could be obtained by this method, but as the present experiments were directed towards the mass infection of plants by many individuals, the method was useless. At the same time it proves of great value when attempting to elucidate the detailed biology of the nematode, a task at present in progress.

Technique.

The *modus operandi* was therefore as follows. Plant material infested with the required strain of nematode was chopped up, dried and incorporated with dried soil. The whole was then spread around the plants in which it was desired to establish the strain and watered. Numbers of these plants were subsequently removed at intervals and examined, first with the naked eye for definite symptoms of attack and then microscopically for the presence of nematodes in the tissues. With the exception of bulbous plants it was found to be essential that the plants were

well established before being exposed to infection. Either plants raised *in situ*, or transplanted seedlings, which had been given ample time to recuperate after moving proved satisfactory. Newly planted material was rarely successfully infected. This is not surprising when one remembers that it is frequently possible to kill the nematode inhabitants of a plant by the simple procedure of removing this from the soil, allowing to wilt and then replanting and watering. The explanation of the phenomenon would appear to lie in the production of some change in the physiology of the plant with which the nematodes are unable to cope.

Usually five cylinders, or pots, were used for each experiment. Nos. 1 to 4 contained the plants into which it was desired to introduce the nematodes, and No. 5 healthy nematode-free plants of the species from which the nematodes had come. Nos. 1, 2 and 5 were infected at the same time. The incidence of the attack in No. 5 indicated the rate at which the nematodes advanced in a favourable host plant, while Nos. 3 and 4 acted as a check on the subsequent activities, if any, in Nos. 1 and 2. In the event of a severe attack developing in No. 5 and no nematodes being found in any plant in the other cylinders, after an interval of 5 or 6 months, the experiment was abandoned.

Fourteen strains of the nematode were used in the course of the experiments, of which no less than 430 were accomplished. Lack of space clearly prohibits the publication of these in extended form, but it is perhaps permissible to figure one in order to indicate the manner in which the records were kept.

Strain	Host provided	Cylinder no.	Date of infection	Examination		
				1 month	3 months	5 months
P. 2	Plantago	16	11. x. 27	*Tylenchus* present	Galls formed	Galls formed
Plantago lanceolata	Narcissus	17	11. x. 27	*Tylenchus* present	Few *Tyl.* present	No *Tyl.* present
	Narcissus	18	Control	No *Tyl.* present	No *Tyl.* present	No *Tyl.* present
Bere Ferrers	Narcissus	19	Control	No *Tyl.* present	No *Tyl.* present	No *Tyl.* present
	Narcissus	20	11. x. 27	*Tylenchus* present	No *Tyl.* present	No *Tyl.* present

Account of strains and summary of results.

A short account of the history of the fourteen strains follows, accompanied by a summary of the experimental results obtained with each. The summarised results show first the name of the plant to which the nematodes were given access. This is followed by a number in brackets

representing the total repetitions of the experiment, which is followed in turn by a brief indication of the findings. Beneath these brief notes are given concerning any points of particular interest which arose in connection with the strain.

Strain F. Host plants oat and cocksfoot.

History. This strain was unusual in that it was equally at home in both oat and cocksfoot grass, *Dactylis glomerata*, occurring abundantly in both in the field at Kingsteignton, Devon, in which it was originally found and able to reproduce freely in either. Negative results had attended previous attempts to infect narcissus and clover with the strain (2).

Experimental results.

	Series A.	
Plantago lanceolata	(8)	No attack
Hypochaeris radicata	(8)	No attack
Dactylis glomerata	(8)	Severe attack
Oat	(4)	Severe attack
	Series B.	
Plantago lanceolata	(8)	No attack
Hypochaeris radicata	(8)	No attack
Dactylis glomerata	(8)	Severe attack
Oat	(4)	Severe attack

Observations. The experiments in Series A were carried out with nematodes which had bred continuously in oat from 1924, when the strain was found, until 1927 when the experiments were made. In view of this, the severe attacks invariably obtained in cocksfoot, after no less than 4 years away from that host, are of particular interest. In Series B the nematodes had spent 4 years in a dry condition in the laboratory immediately prior to the experiments. Nevertheless the virulence of their attack was unabated at the end of the period.

Strain O. 3. Host plant oat.

History. Found at Woodbury, East Devon, in 1927, in an oat field in which the crop had been attacked for two consecutive years. The farm had previously been free and the attack apparently originated from some seed which was traced to a source having a heavy and longstanding infestation.

Experimental results.

Plantago lanceolata	(7)	No attack
Hypochaeris radicata	(7)	No attack
Narcissus	(7)	No attack
Oat	(4)	Severe attack

Observations. Plantain was very abundant in the oat field in which the strain was found, but external and microscopical examination of many plants revealed no signs of attack.

Strain H. 1. *Host plant* Hypochaeris radicata.

History. The strain comprised the entire nematode population of six plants found in an isolated position at Marazion, Cornwall, in March 1928. The finding of these constituted the first record of *T. dipsaci* in this plant in England. In the light of subsequent work it is probable that the infestation in this locality originated in a wind-borne nematode infested seed.

Experimental results.

Plantago lanceolata	(8)	No attack
Narcissus	(8)	No attack
Allium vineale	(8)	No attack
Clover	(8)	No attack
Hypochaeris radicata	(4)	Severe attack

Observations. The locality in which the strain was found was separated from an area in which *Plantago lanceolata* and *P. maritima* were attacked only by a railway track. Nevertheless, no plantain was attacked on the one, or *H. radicata* on the other side of the line, although on both each plant was equally abundant.

Strain H. 3. *Host plant* Hypochaeris radicata.

History. Collected from an extensive infestation on Dawlish Warren, South Devon, in 1928. Judging by the isolated position of the infestation one might well assume that the association between nematode and host was of long standing.

Experimental results.

Narcissus	(12)	Bulbs invariably entered
Plantago lanceolata	(8)	No attack
Allium vineale	(8)	No attack
Hypochaeris radicata	(4)	Severe attack

Observations. Numbers of the worms were found, in each case, to penetrate healthy bulbs. On one occasion extensive decay was caused in 40 per cent. of the bulbs within 3 months of infection. At the same time no eggs were observed to be laid in the host; large numbers of worms died after penetration, and in every case the attack eventually died out. Further, although in many bulbs the decay was palpably caused directly by the presence of the worms in the tissues, this decay was by no means typical of the damage caused by narcissus strains of the nematode.

Strain A. 1. *Host plant* Allium triquetrum.

History. Taken in *A. triquetrum* on St Mary's, Isles of Scilly, in 1928. The plants were growing thickly amongst narcissus in a commercial garden, which were also infested with the nematode.

Experimental results.

Narcissus	(4)	No attack
Allium vineale	(4)	No attack
A. triquetrum	(2)	Severe attack

Observations. Unfortunately only a small supply of material was collected, and so but few experiments were possible. Gibson, of the Isles of Scilly Experimental Station, has since stated that he found no difficulty in inducing this strain to enter and attack narcissus.

Strain P. 1. *Host plant* Plantago lanceolata.

History. Collected from an extensive infestation in the vicinity of the golf course on Dawlish Warren, South Devon. No land was cultivated in the vicinity.

Experimental results.

Narcissus	(17)	No attack
,,	(1)	Bulbs entered
Hypochaeris radicata	(12)	No attack
Allium vineale	(12)	No attack
Plantago lanceolata	(4)	Severe attack

Observations. In one case a number of living and dead nematodes were found in a few of the bulbs 1 month after exposure to infection. No sign of the nematodes was found at the next examination 1 month later.

Strain P. 2. *Host plant* Plantago maritima.

History. Taken on the foreshore at Marazion, Cornwall, and afterwards maintained in *P. lanceolata*. The strain was considered likely to prove of interest owing to the proximity of the locality to large acreages of narcissus grown on a field scale. Further, it has in the past been the practice to cart and dump discarded bulbs, frequently containing *T. dipsaci*, on the tide mark in the immediate vicinity of the infestation. It, therefore, appeared possible that the nematodes might in the first instance have come from narcissus. The experiments suggest that this may indeed have been so.

Experimental results.

Series A.

Narcissus	(6)	Severe attack
Hypochaeris radicata	(6)	No attack
Allium vineale	(6)	No attack
Oat	(6)	No attack
Plantago lanceolata	(4)	Severe attack

Series B.

Narcissus	(6)	Severe attack
Hypochaeris radicata	(6)	No attack
Plantago lanceolata	(4)	Severe attack

Observations. In Series A nematodes entered narcissus freely and after 3 months all stages, including eggs, could be obtained from typical lesions in the leaves. The bulbs were then dried out and used to infect plantain again. Small leaf galls were formed after 1 month and large and typical ones, containing all stages of the worms, after 3 months.

This transference was carried out with a facility so surprising, in view of previous results, that it seemed possible that by some accident a narcissus and a plantain had by some means become mixed. In order to make certain concerning this point a fresh supply of infested *P. maritima* was collected in the original locality and certain of the experiments repeated in Series B. Results identical with those in Series A were obtained. Two months after exposure to infection decay was set up in the narcissus bulbs, this decay being typical of that normally associated with an attack by *T. dipsaci* on this plant.

A portion of this strain has since been kept continuously in narcissus with a view to attempting a transfer back to plantain after a number of years have elapsed.

Strain P. 4. Host plant Plantago lanceolata.

History. Collected from a very extensive infestation in plants growing in a brackish marsh at Bere Ferrers, Devon. The marsh is in most respects extremely isolated, although within half-a-mile of commercial narcissus beds. The infestation was so widespread that it was clearly of very long-standing.

Experimental results.

Narcissus	(7)	No attack
Hypochaeris radicata	(7)	No attack
Oat	(7)	No attack
Plantago lanceolata	(6)	Severe attack

Observations. On no occasion was there the slightest indication of the nematodes entering an alien host.

Strain N. 1. Host plant Narcissus.

History. This strain was known to have bred continuously in narcissus for at least 6 years. Negative results had attended previous attempts (2) to infect oat and clover with the strain.

Experimental results.

Plantago lanceolata	(8)	No attack
Hypochaeris radicata	(8)	No attack
Allium vineale	(8)	Temporary invasion
Narcissus	(4)	Severe attack

Observations. Allium vineale was freely invaded and active nematodes could be found in the tissues one month after infection. No reproduction took place and no trace of the nematodes remained after 3 months.

Strain N. 2. Host plant Narcissus.

History. Another strain definitely known to have been established in narcissus for several, in this case 7, years. Oat and clover had already been exposed to infection (2) and in each of these worms persisted for 3 months, but did not reproduce.

Experimental results.

Hypochaeris radicata	(6)	Temporary invasion
Allium vineale	(6)	No attack
Plantago lanceolata	(6)	No attack
Narcissus	(4)	Severe attack

Observations. The invasions of *A. vineale* in no case persisted for more than 1 month after infection.

Strain N. 3. Host plant Narcissus.

History. Similar in history to strain N. 2. In spite of the long association with one host plant the experimental results are of interest.

Experimental results.

Hypochaeris radicata	(6)	Temporary invasion
Allium vineale	(6)	Temporary invasion
Plantago lanceolata	(6)	(*a*) Wholesale temporary invasion
Narcissus	(4)	Severe attack
(*a*) Narcissus	(2)	(*b*) Severe attack
(*b*) *Plantago lanceolata*	(2)	No attack

Observations. A few dead nematodes were found in the tissues of *H. radicata* 1 month after infection. Wholesale invasion of *A. vineale* took place and, while no eggs were found, living nematodes persisted in the plants for approximately 3 months. In *P. lanceolata* small but definite leaf galls developed 2 months after infection. The galls did not increase in size, no eggs were found, and after 4 months no living nema-

todes remained in the galls or other parts of the plants. Some of the galled material was used (*a*) during the third month to re-infect narcissus. This was successfully accomplished. An attempt to re-infect *P. lanceolata* (*b*) with progeny of these particular worms failed.

Strain N. 4. *Host plant* Narcissus.

History. Known to have bred in narcissus for at least 3 years. Oat and clover had already been exposed to infection (2). Both were entered, nematodes persisting for 3 months but not reproducing.

Experimental results.

Hypochaeris radicata	(8)	No attack
Allium vineale	(8)	No attack
Plantago lanceolata	(8)	Temporary invasion
Narcissus	(4)	Severe attack

Observations. Entry into *P. lanceolata* was effected in some numbers, but no nematodes persisted in the plants for more than 1 month.

Strain N. 5. *Host plant* Narcissus.

History. A strain collected from a narcissus bed in West Cornwall known to have been attacked for 3 years. Other possible host plants in the vicinity were free from attack.

Experimental results.

Hypochaeris radicata	(5)	No attack
Allium vineale	(5)	No attack
Plantago lanceolata	(5)	No attack
Narcissus	(4)	Severe attack

Observations. No single invading nematode was found in any of the three hosts available.

Strain N. 6. *Host plant* Narcissus.

History. Collected from narcissus, bred on in this plant for 2 years and then kept dry in the laboratory for a further period of 3 years.

Experimental results.

Hypochaeris radicata	(6)	No attack
Allium vineale	(6)	No attack
Plantago lanceolata	(6)	Temporary invasion
Oat	(6)	Temporary invasion
Clover	(6)	Temporary invasion
Narcissus	(4)	Severe attack

Observations. Nematodes persisted in those plants temporarily invaded for periods up to 2 months in length. No eggs were found. Attacks on narcissus were exceedingly virulent, even after the enforced quiesence for over 3 years in the laboratory.

Discussion of experimental results.

The striking feature of the whole series is the regularity with which infection of the specific host plant of the strain concerned took place. In no single instance was failure to effect this experienced, even in the experiments made with nematodes which had remained dry in the laboratory for 4 years. The value of this regularity is apparent. It indicates strongly that failure to obtain infection of alien host plants was indeed due to the inability of the nematodes to attack these, and not to any fault in the technique employed.

Turning now to the consideration of the behaviour of the various strains towards the alien host plants we find greatly varying results. Fourteen strains were used. Five of these, namely, O. 3, H. 1, A. 1, P. 4 and N. 5, failed entirely to enter the plants provided, although collectively they had ninety-seven opportunities of so doing. At the same time they did not once, in the twenty possible times, fail to attack their specific host plants. Strain P. 1 is in practically the same category, for, out of forty-six experiments, only in one was a very transitory invasion of a few narcissus bulbs effected. Strain F also apparently ranks with these, with the exception to which attention has already been drawn, namely, that it has remained consistently equally at home on both oat and cocksfoot grass.

In the next category we can place the strains H. 3, N. 1, N. 2, N. 4 and N. 6. These were able to enter one, sometimes two, but never all of the alien hosts provided. The invasions took place with a considerable degree of regularity. For example, H. 3 entered narcissus in each of the twelve experiments carried out with that plant, but on no occasion entered *A. vineale* or *P. lanceolata*. Nevertheless, not one of these strains reproduced in the new host, and the invasions invariably died out after a few months. One point is perhaps not without significance here and that is, the frequency with which nematodes originally occurring in narcissus were able temporarily to invade plantain.

In the final category we have the two strains P. 2 and N. 3, and their behaviour is of sufficient interest to warrant a careful examination of each. Forty-four separate experiments were made with the plantain strain P. 2. Twelve of these involved the use of narcissus as the alien host, and in each case an attack, typical of that usually found on narcissus, was set up, reproduction occurring freely. Attention has already been directed to the fact that the locality from which the strain was originally taken is one in which contact between plantains and

nematode-infested bulbs has probably taken place over a considerable number of years. Finally, in spite of the readiness with which interchange between these particular hosts was effected, entirely negative results attended attempts to infect three other potential hosts of *T. dipsaci*.

Strain N. 3, in spite of at least 7 years' continuous breeding in narcissus, readily effected temporary invasion of various other plants. It was able to persist in plantain for 3 months and at the end of that time to set up a fresh infestation when returned to narcissus. Thus both strains in this last category, as did those in the previous one, give some indication of an affinity between narcissus and plantain feeding strains.

Conclusions.

Consideration of the experimental data set forth above indicates that much remains to be discovered concerning the factors governing the formation and maintenance of the biologic strains of *T. dipsaci*. In these experiments no provisions were made for the nematodes to select their favourite host from a number of species known to be susceptible to attack. Instead they were given access, without alternative, to a single potential host of the nematode, other than the one on which they were known to have been feeding previously. Time after time failure to invade the plants provided occurred, thus indicating that the biologic strain is a very real thing. Certain strains were to some extent adaptable, in that they entered alien hosts, but they were unable to persist themselves or to reproduce in them. Finally, two of the strains used were found to enter and to reproduce alternately in such dissimilar plants as the narcissus and the plantain.

At first sight one might be inclined to label these strains as rigid, less rigid and adaptable, but any such categorising without first embarking upon an almost infinite number of experiments is not permissible. For example, strain H. 1, which in the present series of experiments attacked nothing but its original host, *H. radicata*, might well prove to be quite at home in phlox, teazle or any other of the 130 known hosts of the nematode, which number is constantly being added to. Nevertheless, the present series of experiments taken in conjunction with those described previously (2) are sufficient in number to warrant the assumption that strains are not able habitually to transfer from species to species of host at random. If further proof of this be needed, it is provided by the enormous number of field observations which have been made from time to time to the same effect, and to cultural practices carried out, particularly in Holland, which would be quite out of the question were wholesale transfer from host to

host to occur with any frequency. At the same time it has been shown that strains attacking two, or perhaps more hosts, do occur with some frequency, and it is not without significance that one strain which had, to the writer's knowledge, bred continuously in narcissus for 7 years, was able at the close of that period to enter and form galls upon plantain.

We turn now to the purely practical aspect of the problem, for this was the purpose for which the work was initiated. It is disturbing to find that strains of *T. dipsaci* attacking both weeds and cultivated plants are by no means uncommon. The oat-cocksfoot combination is a dangerous one from the point of view of the general farmer. Actually this has only been found in two adjacent fields in South Devon, which have subsequently been put down to permanent pasture, but there is little reason to doubt the existence of similar combinations elsewhere. The narcissus-plantain strains are also of no little importance in the commercial narcissus-growing areas in the west of England. The bulb represents the growers' capital and fortunately can be freed from the attack of *T. dipsaci* by use of the hot-water bath. A combination of this treatment and a period of 3 years under another crop for the affected ground will normally eradicate the nematode. Where the strain concerned is one capable of tiding over that period in plantain, re-infection of the next bulb crop is bound to take place immediately on planting.

It must not be inferred from the above that every weed infestation is an active menace to a cultivated crop, should this happen to be a potential host of *T. dipsaci*. Both *P. lanceolata* and *H. radicata* are frequently infested in the west of England, usually without causing harm to cultivated plants in the vicinity. At the same time the experimental evidence indicates clearly that such transferences are able to take place. That this does sometimes occur is suggested by the frequency with which narcissus bulbs over a wide area may become nematode infested, in spite of every precaution having been taken against contamination from other bulbs.

At the present state of our knowledge it can only be suggested that, where infestation of crops by *T. dipsaci* occurs, attention should be paid to the strain of the nematode involved. Nearby crops and weeds should be examined for the presence of the nematode, and a simple series of experiments might be carried out with potential host plants in the vicinity. The results of these would give some indication as to the likelihood or not of the infestation being local in origin. If this latter proves, as it sometimes will, to be the case, it is clear that more drastic measures than the usual rotational method will have to be employed. Thorough weed eradication and bare fallowing would probably have to be pursued

for some seasons before the affected crop could again be grown with a reasonable degree of safety.

SUMMARY.

1. The significance of the occurrence of biologic strains in *T. dipsaci* is discussed and reference made to previous work on the subject.

2. The technique by which the nematode may regularly be induced to set up an infestation in a favoured host plant is described, as is its application to the experiments made.

3. Experimental results obtained when fourteen strains of the nematode, of known history, are given facilities for entering alien host plants are presented in detail.

4. The conclusions arrived at are, that biologic strains of *T. dipsaci* occur, but that these strains are in some cases able to attack more than one host plant. Further, some affinity appears to exist between strains occurring in the narcissus and in the common plantains.

5. The practical application of our knowledge, in the event of a crop being attacked by the nematode, is discussed.

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THE INFLUENCE OF ANTISEPTICS ON THE BACTERIAL AND PROTOZOAN POPULATION OF GREENHOUSE SOILS

PART I. NAPHTHALENE

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(With 10 Text-figures.)

Introduction.

The discovery of the phenomenon of the increased growth of plants in soil treated either with heat or with antiseptics has led to the formulation of many theories concerning its cause. The best known and most widely discussed of these is the theory of partial sterilisation brought forward by Russell and Hutchinson (1909, 1913). These investigators found that the numbers of bacteria in the soil rose when doses of antiseptics which killed the protozoa were applied, and concluded that the beneficial effects of antiseptics were due in part to the ammonification of these dead protozoa by bacteria, and in part to the greater activity of the bacteria in decomposing the organic matter of the soil when the inhibiting effect of the protozoa had been removed. The work of Russell and Hutchinson was largely carried out on field soils, and attempts were made to remove the antiseptics by volatilisation, after sufficient time had been allowed for the production of their full effect. A. Matthews (1923) has used greenhouse soils, in which the speed of events is much greater than in field soils, and has allowed the antiseptic to remain in the soil during the experiments. The results showed that the high bacterial content produced by antiseptics was not permanent, as was previously believed to be the case, but transitory, the number of bacteria falling gradually and continuously towards that in an untreated control soil. Also the bacterial numbers were apparently independent of the presence or absence of protozoa, some of the antiseptics used not being toxic towards them. Finally, since the effect of various substances on the bacterial content of the soil varied in the same direction as does the heat of combustion of these substances, Matthews argues that the increase in bacterial numbers is a question

mainly of nutrition of the bacteria, and would attribute the increased fertility of a soil produced by partial sterilisation to the activity of the bacteria in breaking down the organic matter of the soil. Skinner (1926) severely criticises Matthews' views on the ground that such organisms utilising non-nitrogenous food material must, of necessity, be in competition with the crop for the available supply of nitrogen. He considers, with Waksman and Starkey (1923), that the fungi play at least as important a part as the protozoa in the phenomena of partial sterilisation. That aromatic hydrocarbons and their derivatives can act as sources of energy for bacteria has been suggested by various workers; for example, by Buddin (1914, *a*), Sen Gupta (1921), Matthews (1923) and Tattersfield (1927). Sen Gupta has isolated bacteria capable of decomposing phenol and *m*-cresol, and Tausson (1927) has described certain organisms which can utilise naphthalene as their sole source of carbon. Gray and Thornton (1928, *a*) have carried out an extensive investigation, in which the widespread occurrence of bacteria capable of utilising phenol, *m*-cresol, naphthalene, and other allied compounds was indicated, and many species of these bacteria were isolated and characterised.

The work described below has been designed to investigate more closely the action of antiseptics on the bacteria and protozoa of greenhouse soils, and it has been found desirable to confine attention to one antiseptic only, namely, naphthalene.

METHODS.

Owing to the difficulty of obtaining samples of fresh greenhouse soil at short notice it was found convenient to use air-dried soil for this work. The soil selected was cucumber soil obtained from the Research Station at Cheshunt through the courtesy of Dr W. F. Bewley, to whom the author's best thanks are due. As soon as received, the soil was spread out to dry for a few days, passed through a 3 mm. sieve, and stored in an earthenware tank covered with brown paper to exclude dust. When used for experiments, the soils have been brought to their optimum water content, which has been taken as 50 per cent. of the maximum water-holding capacity as determined by Hilgard's (1906) method. Four separate samples of soil have been under investigation. The first had an optimum water content of 40 c.c. per 100 gm. of dry soil, and the second, one of 28 c.c. Both of these soils had come straight from the beds and were in good condition. The third sample was from a heap of "sick-soil" which had been standing for 2 years, and had become covered with weeds. The optimum water content of this sample was 40 c.c. per 100 gm. of

dry soil. The fourth sample was another fresh soil in good condition: optimum water content 50 c.c. per 100 gm. dry soil.

Method of treating the soil with naphthalene. Buddin's notation (Buddin, 1914, *a*) has been adopted to express the amount of naphthalene added to the soil, *i.e.* an "$M/10$ dose" implies that an amount of naphthalene equivalent to one-tenth of a gram-molecule per kg. of dry soil has been added. The required amount of naphthalene was weighed out, added to the soil, which was spread on a sheet of brown paper, and mixed with the soil as rapidly and as uniformly as possible. (Unsterilised "flake" naphthalene was used for this purpose.) Some slight loss by volatilisation may have occurred during this process. When soils received a further addition of naphthalene, this procedure was not possible, so the weighed amounts of naphthalene were added to the soil in each flask, and stirred in with a sterile glass rod, any soil adhering to the rod being returned to the flask. For this purpose, in order to avoid the entry of fresh organisms, the naphthalene was sterilised by heating it to boiling point (218° C.) for 10 minutes in a sterile glass dish 1½ in. deep and fitted with a lid. When the naphthalene had solidified it was ground to a powder in a sterile glass mortar and transferred to a sterile weighing bottle. It was found that the powder thus obtained was not so fine as that obtained from the original "flake" naphthalene, and may not have been so readily attackable by bacteria.

Method of setting up experiments. The dry naphthalened soil was weighed out, in portions containing 100 gm. of soil (allowance being made for the naphthalene content), into sterile 500 c.c. conical flasks which were closed with cotton-wool stoppers. All the flasks were of similar size and shape and the stoppers were as uniformly tight as possible. Sterile distilled water was then added to bring the water content to the optimum value, and stirred in with a sterile glass rod, any soil adhering to the rod being returned to the flask. The soil was left in a loose state and the flasks were shaken gently to level the surface of the soil. The flasks were kept at 20° C. The loss of water by evaporation was found to be negligible, any water condensing in the upper part of the flasks during incubation being stirred into the soil before taking samples for counting.

Matthews (1923) and Tattersfield (1927) have shown that the bacterial numbers and the rate of disappearance of naphthalene in the soil are both influenced by the amount of aëration. Continued sampling from a quantity of soil in a bottle involves the admission at each sampling of a volume of air which is by no means negligible, and therefore it follows that the successive counts will be subject to increasing amounts of

aëration. This admission of air has been avoided by setting up, in each experiment, as many flasks as there were counts to be made, the soil in each flask being used for one count only. Consequently the only factor to be considered in comparing counts made by this method (except the factors of the accumulation of metabolic products and the diffusion of air through the stoppers) is the time of incubation of the soil. The influence of the accumulation of products of metabolism will be greater in this method than in the usual method of storing the treated soil, because of the smaller aëration, but it will not be irregularly affected. The factor of the diffusion of air through the stoppers remains unaltered. It is realised that the effects of stirring soil containing an accumulation of the products of decomposition of naphthalene are not the same as those of stirring untreated soil, but whenever certain flasks in an experiment received an additional quantity of naphthalene, all the other flasks were also stirred in order to neutralise these effects as far as possible.

Method of making bacterial counts. Since it became apparent at once that the fluctuations in the bacterial numbers were very rapid, counts have been made at intervals of 24 hours, the plating method being used for the majority of the counts. Previous workers have employed nutrient gelatine for making the dilution plates, but in this investigation Thornton's medium has been employed (Thornton, 1922) as it has the following advantages. (1) It is perfectly standard and easily reproducible. (2) It approximates more nearly than nutrient gelatine to the conditions obtaining in the soil. (3) It avoids trouble due to liquefying organisms. (4) It restrains the growth of spreading organisms. (5) It reduces the development of fungi to a minimum. All plates were incubated for 7 days at 24° C. In the earlier experiments the whole of the soil in any one flask was used for a count, 200 c.c. of sterile tap water being added and the flask shaken for 5 minutes before further dilutions were made. In later experiments, where samples were required for other purposes as well, the method adopted was first to turn the soil out on to a sheet of sterile brown paper, mix it rapidly, and return it to the flask. 10 gm. were then weighed out into 250 c.c. of sterile physiological salt solution and shaken for 5 minutes before making further dilutions. Plates were made in quintuplicate from at least two dilutions, in order to obtain a suitable colony density per plate. In counting the colonies a dissecting microscope was used ($\times$ 8). The plates were divided into strips by thin ink lines drawn on the back, and by moving up one strip and down the next, and so on, the total number of colonies on each plate was found without difficulty. The probable error is taken to be plus or minus 5 per cent. of

the final figures, which were calculated as millions of bacteria per gm. of dry soil. In some experiments an attempt was made to enumerate the "naphthalene-decomposing bacteria" by making counts on a medium containing no other source of carbon but naphthalene. No precautions were taken to remove dissolved carbon dioxide, or to incubate the plates in an atmosphere free from this gas. Valley and Rettger (1927) have shown that the removal of all traces of carbon dioxide from an otherwise suitable medium exerts an adverse influence on bacteria growing therein. The medium employed was based on the solutions for the isolation of naphthalene-decomposing bacteria employed by Gray and Thornton (1928, *a*), and had the following composition:

Distilled water	1000 c.c.	$FeCl_3$	0·02 gm.
K_2HPO_4	1·0 gm.	$(NH_4)_2SO_4$	0·50 ,,
$MgSO_4$	0·2 ,,	KNO_3	0·50 ,,
NaCl	0·1 ,,	Naphthalene	10·00 ,,
$CaCl_2$	0·1 ,,	Agar	15·00 ,,

The inorganic salts were dissolved in the water, the reaction brought to *p*H 7·3, the naphthalene and agar added, and the whole heated in the autoclave for 15 minutes at 15 lb. pressure. The hot liquid was then shaken whilst cooling, so that the naphthalene solidified in small crystals, to ensure that, on filling into test-tubes, the substance became approximately equally apportioned between the tubes. The medium was then sterilised for 15 minutes at 15 lb. pressure. Before use of the medium for dilution plates it was melted in a bath of boiling water, and after cooling to about 80° C., the solidifying point of naphthalene, the tubes were removed from the bath and shaken in order to obtain the naphthalene in small crystals. After the formation of these the tubes were returned to the water bath and allowed to cool to 42° C., at which temperature the plates were poured. In spite of these precautions the naphthalene often solidified in ball-like masses, thus affecting the distribution of the naphthalene in the plates.

In the last two experiments counts were made by a direct method devised by Gray and Thornton (1928, *b*). Full details have not yet been published, but the writer is indebted to the authors for a complete description of the method as at present employed at Rothamsted. Briefly, this consists in shaking a weighed quantity of soil with a given volume of a suspension of indigo particles, the number of particles per c.c. of the latter having previously been counted by use of a Thoma haemacytometer. Spots of the soil suspension are then made on clean slides and

allowed to dry evenly. The bacteria are stained, first with carbol erythrosin and second with aqueous erythrosin, washed, and dried. The slides are examined with the $\frac{1}{12}$th oil immersion objective and No. 10 eyepiece, and the ratio of bacteria to indigo particles found by counting the number of each in several fields. The true number of bacteria per gm. of soil is calculated from this ratio, the concentration of soil and indigo particles being known. Owing to the thickness of the haemacytometer coverslip available, the writer found it impossible to use the oil immersion objective for counting the particles in the indigo suspension, a Watson $\frac{1}{6}$th objective being used. This probably means that a low count of indigo particles has been obtained, some of the smaller ones having been missed. However, since the same suspension was used throughout an experiment, all counts will need to be multiplied by the same factor, and so, although the figures given may actually be low, the results will not be affected.

Method of counting protozoa. The protozoa were counted by Cutler's dilution method (Cutler, 1919; Cutler, Crump, and Sandon, 1922). The soils used contained a large excess of lime, so, in order to avoid reduction of the strength of the acid used for the cyst counts to a figure much below 2 per cent., 2·4 per cent. HCl was employed. 75 c.c. of acid of this strength were added to 10 gm. of wet soil, and the resulting strength of the acid was found to be just below 2 per cent. An inherent difficulty in the method is that small fluctuations in a large number of protozoa cannot be detected and, since the numbers of protozoa found in this investigation have usually been high, it follows that only the larger fluctuations have been recorded. Counts have been made of amoebae and flagellates: ciliates were so few in number that they have not been considered.

Method of estimating naphthalene. Naphthalene was estimated by the method of distillation in steam into alcoholic picric acid devised by Tattersfield (1927). The only difference was in the amount of soil taken for the estimation. The method is not capable of dealing with quantities of naphthalene much in excess of 100 mgm. without employing very large absorbers and greatly increasing the time of distillation; therefore, during the early stages of the experiments, when the quantity of naphthalene present was large, 10 gm. of soil were used for the distillation, this quantity being increased to 50 gm. when the quantity of naphthalene had fallen sufficiently. This introduces a sampling error, and involves a multiplying factor of 13 to 14 when 10 gm. of soil are used, the factor varying with the moisture content of the soil; hence the error in the larger amounts can hardly be less than 50 mgm., and may be greater.

The control distillations on the untreated soil gave negligible differences in titration.

Method of measuring the pH *of the soil.* Owing to pressure of work a very simple and not very accurate technique was employed. 10 gm. of soil were shaken for 5 minutes with 50 c.c. of distilled water and filtered. The *p*H of this 1 : 5 extract was measured by Gillespie's colorimetric method (Gillespie, 1920), using brom-thymol-blue as indicator. Differences of less than 0·2 are probably not significant.

Method of estimating ammonia. Ammonia was estimated by the aëration method, the apparatus and technique introduced by D. J. Matthews (1920) being employed. The alkaline agent used was 25 per cent. sodium chloride plus ignited magnesia, in order to avoid decomposition of the unstable nitrogenous material which is present in greenhouse soils. Aëration was carried out for a period of 5 hours with an air current of approximately 300 litres per hour. 10 gm. of soil were used for each determination.

Experimental.

Exp. I. In this experiment, cucumber soil, of optimum water content 40 c.c. per 100 gm. of dry soil, was treated with an $M/10$ dose of naphthalene in the manner described above. Fourteen flasks were prepared, and the bacteria were counted daily for the first 7 days, and again on the ninth and fourteenth days. The five remaining flasks were left untouched until the results obtained from the first counts had been considered. The data obtained from these counts are given in Table I and are shown graphically in Fig. 1.

Table I.

The effect of an M/10 *dose of naphthalene on the bacterial content of cucumber soil.*

Days after addition of naphthalene	Bacteria in millions per gm. of dry soil		Presence of naphthalene
	Treated	Untreated	
Initial	8·4	8·4	—
1	862	—	Strong odour. Crystals visible
2	3162	—	Strong odour. Crystals visible
3	3180	59·4	Distinct odour. No crystals
4	944	—	Faint odour
5	579	—	Very faint odour
6	419	—	Very faint odour
7	436	—	Very faint odour
9	438	102·6	No odour
14	290	—	No odour
15	—	74·0	—

It will be seen that the numbers of bacteria rose with great rapidity to a maximum value of 3180 millions, the high numbers being maintained for 24 hours, after which, on the fourth day, they fell suddenly and continued to decrease till the sixth day, when a steady value of just over 400 millions persisted till the ninth day. Counted again on the fourteenth

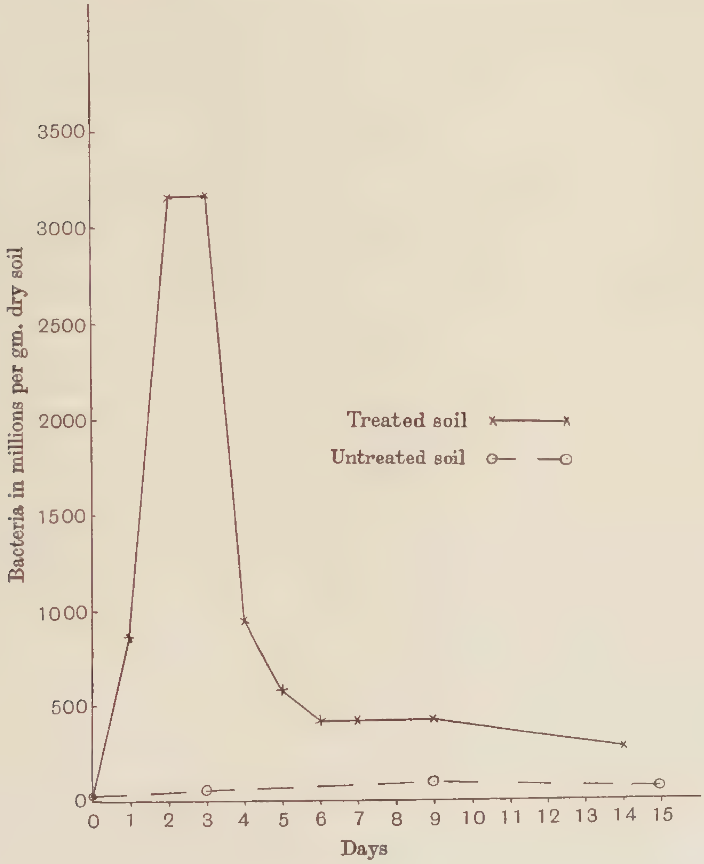

Fig. 1. **Bacteria in cucumber soil treated with an $M/10$ dose of naphthalene.**

day, the numbers were found to have declined further, but were still well above those of the controls. As will be seen, the controls were counted less frequently than the treated flasks, but such figures as were obtained indicate a steady rise and fall reaching maximum numbers some days later than was the case in the presence of naphthalene. The smell of the naphthalene had almost gone by the fourth day, but traces persisted for

several days afterwards. The form of the curve showing the number of bacteria in the treated soil at once suggests that the naphthalene was being used as a source of energy by the bacteria, the bacterial numbers falling rapidly as soon as the naphthalene had been used up. The possibility that the increase in numbers was wholly due to the bacteria utilising the accumulation of dead organisms produced by air-drying the soil is ruled out by the fact that the control, which contained an equally large accumulation, shows no such large increase in numbers, the increase here being relatively unimportant and much slower. For a similar reason the physical and chemical effects of air-drying the soil cannot be the cause of the enormous increase in bacteria. Two further possibilities remain, first that the naphthalene had rendered the organic matter of the soil much more readily available, and second that the naphthalene had either killed the protozoa, or temporarily prevented them from developing. With the object of testing the second of these possibilities the five remaining flasks were given a second $M/10$ dose of naphthalene, and counts made as before. The results are shown in Fig. 2.

The second dose produced a smaller increase in numbers than the first, but after this second addition the subsequent fall was more gradual. The explanation of this may be that the second dose of naphthalene was decomposed more slowly than the first, being, as stated previously, in a coarser state of division. Again, the multiplication of the bacteria may have been hindered by the presence of metabolic products of the bacterial activity resulting from the first addition of naphthalene. Obviously these considerations prevent one from drawing definite conclusions as to the part which the protozoa have played, but the regularity of the rise and fall of the bacterial numbers strongly suggests that active protozoa were not present, at any rate not in large numbers.

Exp. II. The soil in this experiment was weighed out in 100 gm. portions into 500 c.c. conical flasks, moistened to the optimum water content and incubated at 20° C. for 3 days in order to develop the protozoa, before being treated with naphthalene. The results are shown in Fig. 3.

As will be seen later (see Exp. VI) 3 days' incubation at 20° C. is amply sufficient to develop a good trophic protozoan fauna in the soil. Comparison of Fig. 3 with Fig. 1 shows that the bacterial increase follows the same course, irrespective of the presence or absence of active protozoa in the soil at the time of treatment, unless the lower count on the third day be regarded as evidence that the protozoa are depressing the numbers of bacteria. Against this it may be remarked that the count is higher on

the fourth day in Exp. II than in Exp. I, so that we may also regard the lower count on the third day as evidence of a rather slower rate of utilisation of the naphthalene by the bacteria, and not as evidence of the activity of protozoa. The figures obtained so far indicate that naphthalene has a depressing effect on the active protozoa.

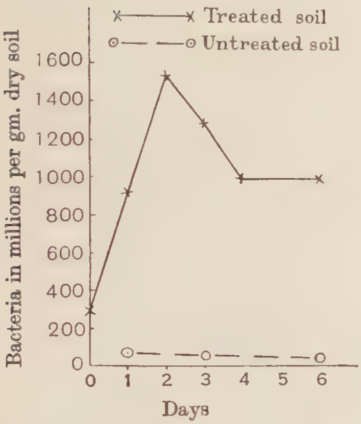

Fig. 2. Bacteria in cucumber soil treated with a second $M/10$ dose of naphthalene.

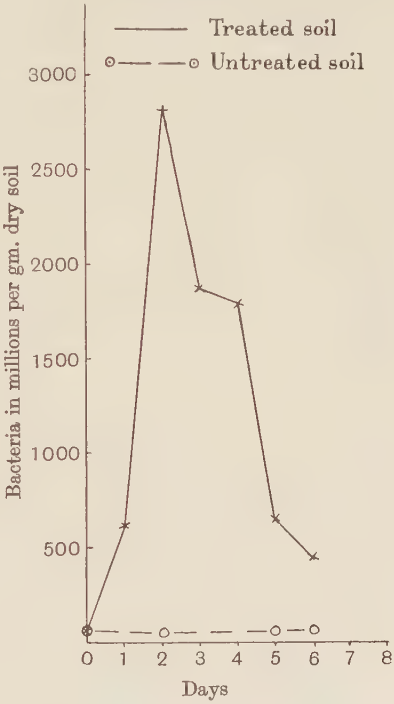

Fig. 3. Bacteria in cucumber soil incubated for 3 days at 20° C. before the addition of an $M/10$ dose of naphthalene.

Exp. III. In this experiment seventeen flasks were prepared, and counts made daily for 5 days. At the end of this period the remaining flasks were divided into two sets; one of these was kept in the incubator, while the soil in the other set was turned out and dried for 10 days. The soil in the untouched flasks and the dried soil were then re-treated with naphthalene and counts continued. The object of this experiment was to determine the influence of the presence or absence of protozoa on the increase in bacterial numbers produced by a second dose of naphthalene. The dried soil would not contain active protozoa, while the undried soil presumably would, unless the protozoa had all been killed by the first

dose of naphthalene. The results are shown in Fig. 4. The curves indicate clearly that the bacterial numbers produced by a second dose of naphthalene are approximately the same whether the soil has been dried between the doses or not; *i.e.* that the increase in numbers of bacteria is apparently independent of the presence or absence of active protozoa when the second dose is added. However, the curves in Fig. 4 are not of similar shape to that in Fig. 2, and it is difficult to account for the low numbers on the seventeenth, eighteenth and nineteenth days in Exp. III unless we suppose a rapid increase in the numbers of protozoa at this

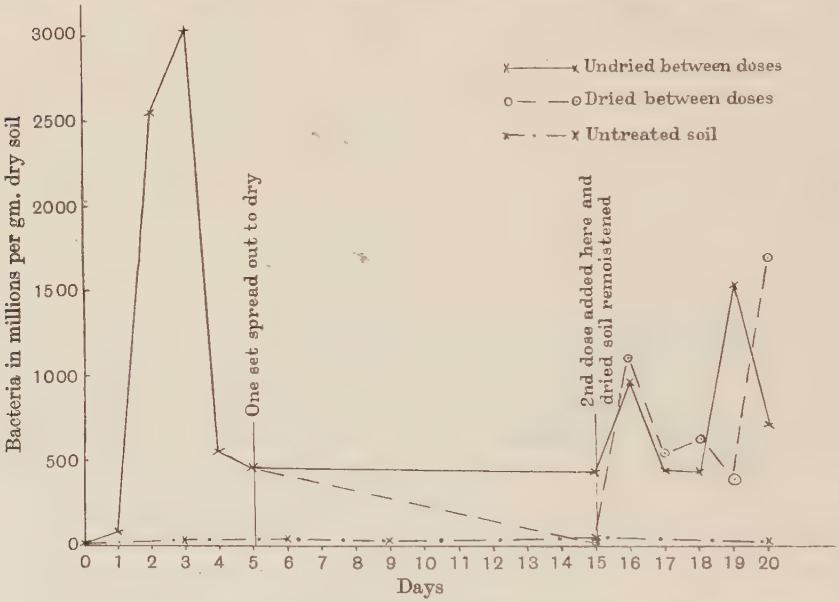

Fig. 4. Bacteria in cucumber soil dried and undried between doses of naphthalene.

time, followed by an equally rapid decrease. As will be seen later (see Exps. VII and VIII), soil which has been treated with naphthalene subsequently contains a large number of protozoa which are mainly in an inactive condition except during certain short periods in which excystation and multiplication take place. The bacterial numbers obtained as a result of the addition of a second dose of naphthalene will depend upon the condition of the protozoa at the time of this second addition, since, if it is made just after a period of protozoan activity, the bacterial multiplication will be able to proceed unchecked, as in Exp. I (Fig. 2). If, on the other hand, the addition is made a day or two before a period of protozoan activity is due, sufficient of the naphthalene

may have been removed in this interval to allow the protozoa to excyst and produce a depression in the bacterial numbers, as in Exp. III (Fig. 4).

The experiments described so far were somewhat unsatisfactory in that one had no certain knowledge of the presence or absence of active protozoa. Therefore it was decided to repeat some of the experiments, making counts of protozoa as well as of bacteria, and for the purpose of these experiments the second sample of the above-mentioned soils was used.

Exp. IV. The action of naphthalene on soil protozoa was first investigated qualitatively. 100 gm. of air-dry soil was treated with an $M/10$ dose of naphthalene, brought to its optimum water content of 28 c.c. per 100 gm. of dry soil, and incubated at 20° C. Daily samples of 1 gm. were taken, inoculated into 20 c.c. of sterile soil extract in small dishes, and kept at 20° C. The cultures so obtained were examined after 2 days, and again after 8 days, for the presence of protozoa. The results are given in Table II.

Table II.

The effect of an M/10 *dose of naphthalene on the development of protozoa in cucumber soil.*

Day on which sample was taken	Results after 2 days' incubation of sample						Results after 8 days' incubation of sample					
	Control soil			Treated soil			Control soil			Treated soil		
	C.	F.	A.	C.	F.	A.	C.	F.	A.	C.	F.	A.
1st	+	+	+	0	0	0	+	+	+	+	+	+
2nd	+	+	+	0	0	0	+	+	+	+	+	+
3rd	+	+	+	+	+	+	+	+	+	+	+	+
4th	+	+	+	+	+	+	+	+	+	+	+	+

C. = ciliates; F. = flagellates; A. = amoebae.

N.B. Microscopical examination showed that there was excellent bacterial growth in all cultures.

The results show that the cultures made from samples taken on the first and second days contained no protozoa after 2 days' incubation, whereas cultures made from samples taken on the third and fourth days did contain protozoa. The explanation would seem to be that, in contrast to samples 3 and 4, though amply supplied with food for protozoa as was shown by microscopic observation of the bacterial development, samples 1 and 2 contained an amount of undecomposed naphthalene sufficient to suppress the development of these larger organisms. The correctness of this explanation is borne out by the results of the examination after

8 days' incubation of the samples, when all cultures contained protozoa, and culture No. 1 alone had a faint odour of naphthalene. The disappearance of naphthalene from the cultures during this period, whether by bacterial decomposition or by volatilisation, had obviously resulted in the production of conditions suitable for the development of the protozoa. We may, therefore, conclude that an $M/10$ dose of naphthalene will suppress the development of protozoa for at least 2 days when added to air-dried cucumber soil.

Exp. V. For this experiment nine flasks were set up, and counts of bacteria and protozoa made daily, except that counts of protozoa were omitted on the first two days, the previous experiment having shown that these latter organisms are inactive during this period. The results are given in Table III and are shown graphically in Fig. 5.

Table III.

The effect of an M/10 *dose of naphthalene on the numbers of bacteria and protozoa in cucumber soil.*

Days	Bacteria in millions per gm. dry soil	Active protozoa per gm. wet soil		Total protozoa per gm. wet soil	
		Amoebae	Flagellates	Amoebae	Flagellates
0	0·25	—	—	—	—
1	713	—	—	—	—
2	3200	—	—	—	—
3	1229	395	0	450	1,800
4	1482	5,245	0	5,300	2,600
5	1446	10,680	1,700*	11,000	2,600
6	1388	10,550	3,250	11,000	3,700
7	917	102,000†	35,100†	110,000†	36,000†
8	904	92,000†	10,350	110,000†	11,000
9	1505	6,300	0	7,600	1,800

* This figure is not significant.

† These figures are too low, the series of dilutions employed not being sufficiently high.

It appears from the data given in Tables I and III that this sample of soil can support a larger bacterial population for a greater length of time than the first sample. It will be seen that the bacterial numbers first rise rapidly and then fall to a fairly steady value of about 1400 millions per gm., and that during this period the active amoebae are not present in any large numbers. It is thus clearly shown that the first fall in numbers of bacteria is not due to the action of active amoebae. Then follows a period of great activity of both amoebae and flagellates, coinciding with a depression in the bacterial numbers, the latter rising again as soon as the numbers of active protozoa have diminished. It thus appears that the action of naphthalene on the soil amoebae and flagellates

is to retard their development. After the disappearance of the naphthalene from the soil, the protozoa are able to develop abundantly and depress the numbers of bacteria. Further, since the fall in bacterial numbers which follows the initial rise occurs before there is any great

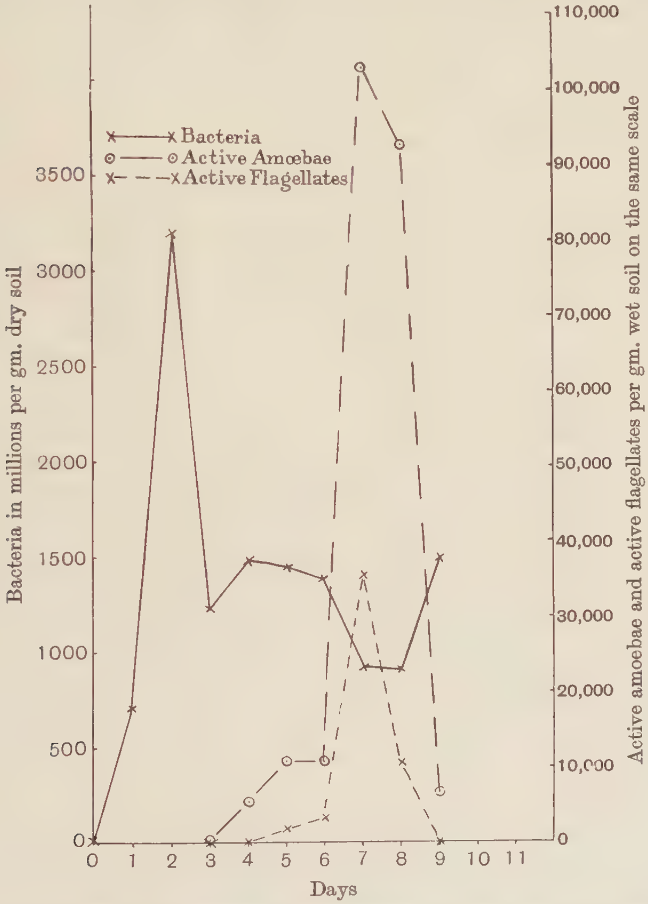

Fig. 5. Bacteria, active amoebae and active flagellates in cucumber soil treated with an $M/10$ dose of naphthalene.

activity of protozoa, it would seem that the naphthalene was acting as food for the bacteria, the fall in numbers being due to the exhaustion of this source of energy. It is probable that the coincidence of the increase in activity of flagellates with the depression in bacterial numbers is only a chance one, as it is well known that the effect of flagellates on the

bacteria is small compared with that of the amoebae. Comparison of the figures for the total with those for the active amoebae in the soil shows

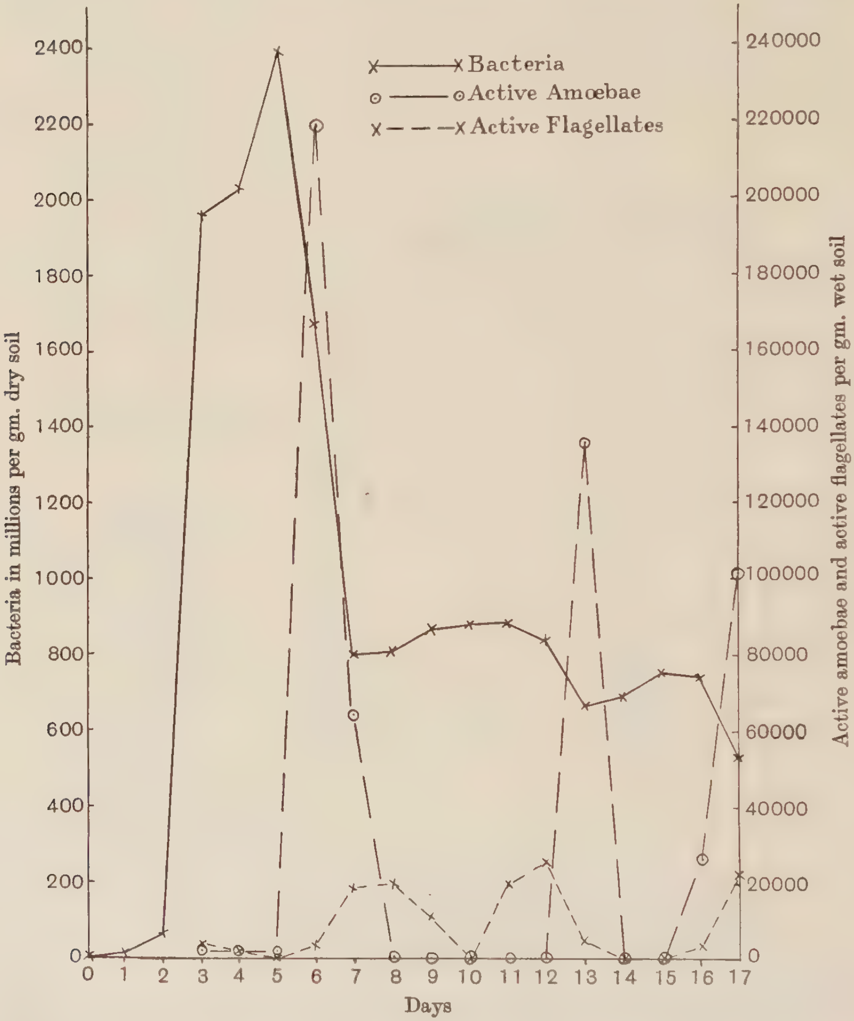

Fig. 6. Bacteria, active amoebae and active flagellates in cucumber soil treated with an $M/10$ dose of naphthalene.

that the increase in numbers of active amoebae is due mainly to multiplication and not to excystment, and that the fall in these numbers is due to their death, and not to cyst formation.

Exp. VI. In order to show definitely that naphthalene suppresses development of the soil protozoa, a count of protozoa was made on untreated soil after three days' incubation, and the results compared with those previously obtained in the treated soil (see Table IV). The results show that there has been a much greater development of both amoebae and flagellates in the untreated soil, thus confirming the results obtained in Exps. IV and V.

Table IV.

Counts of protozoa after 3 days' incubation of treated and untreated soils.

Active protozoa per gm. of wet soil				Total protozoa per gm. of wet soil			
Untreated soil		Treated soil		Untreated soil		Treated soil	
A.	F.	A.	F.	A.	F.	A.	F.
5,500	22,100	395	0	11,000	23,000	450	1,800

A. = amoebae; F. = flagellates.

Exps. VII and VIII. In Exp. VII, in order to investigate the relationship between bacteria and protozoa after a greater length of time, the soil was treated with naphthalene and incubated for 8 days before counts of bacteria and protozoa were started, the first seven counts being omitted in order to economise in time and labour. In Exp. VIII a complete series of counts has been made for a period of 17 days. The results of Exp. VII are given in Table V, and of Exp. VIII in Table VI and Fig. 6. In Exp. VIII bacterial counts were also made on the naphthalene medium described above. These results are not given in Table VI, but have been reserved for consideration under the heading of Exp. X.

Table V.

The effect of an M/10 *dose of naphthalene on the numbers of bacteria and protozoa in cucumber soil.*

		Protozoa per gm. of wet soil			
		Active protozoa		Total protozoa	
Days	Bacteria in millions per gm. dry soil	Amoebae	Flagellates	Amoebae	Flagellates
8	925	15,200	18,800	59,200	28,800
9	800	0	20,000	176,000	41,000
10	864	0	10,800	41,000	20,800
11	813	15,200	21,500	59,200	28,800
12	659	0	0	28,800	41,000
13	678	26,600	0	121,600	84,800
14	506	0	10,800	28,800	14,400
15	512	0	0	14,400	20,800

Table VI.

The effect of an M/10 *dose of naphthalene on the numbers of bacteria and protozoa in cucumber soil.*

Days	Bacteria in millions per gm. dry soil	Protozoa per gm. of wet soil: Active protozoa: Amoebae	Active protozoa: Flagellates	Total protozoa: Amoebae	Total protozoa: Flagellates
1	6·4	—	—	—	—
2	109	—	—	—	—
3	1950	1,260	3,450	1,300	5,300
4	2019	900*	1,050†	900*	3,700*
5	2384	1,300*	0	1,300*	900
6	1667	218,500	3,250	230,000	5,100
7	790	63,800	18,200	84,800	20,800
8	801	0	19,500	59,200*	20,800
9	861	0	10,800	121,600	14,400*
10	874	0	0	59,200	14,400
11	874	0	19,000	28,800	20,800*
12	835	0	25,200	14,400	28,800*
13	659	135,000	4,000†	176,600	14,400
14	685	0	0	41,000	10,400*
15	749	0	0	41,000	20,800
16	733	25,600	3,200†	41,000	10,400
17	522	100,800	21,600	121,600	20,800

* These figures do not differ significantly from the preceding figure.
† These figures are not significant.

The figures for the bacteria show that, after the first large rise and fall, the numbers of bacteria decrease by steps. For instance, in Exp. VII, the numbers show a sudden significant decrease on the ninth day, the twelfth day, and the fourteenth day, the numbers remaining constant or even showing a tendency to rise in the intervals. These sudden decreases are in each case preceded by an increase in activity of the amoebae. Flagellates show a periodicity also, but not so closely connected with the bacterial numbers. The figures for the active amoebae in this experiment are, however, barely significant. In Exp. VIII we see the same phenomenon. The bacteria first rise and then fall, and thereafter show sudden significant decreases on the thirteenth and seventeenth days, these coinciding with periods of marked activity of the amoebae. The figures for the active amoebae are significant in this experiment. Flagellates show periodic activity, but again these periods are not so closely connected with the bacterial numbers. Contrary to the result of Exp. V, the first large decrease in bacterial numbers in Exp. VIII is apparently associated with the first period of activity of the amoebae, but it will be noticed that the bacterial numbers have not risen so high, and the numbers have been maintained for a longer period before falling. The

increase in activity of the amoebae has, on the other hand, occurred rather earlier. It appears that the less intense, but more prolonged, activity of the bacteria is a result of the changes produced by storing the soil in the air-dry condition for a considerable time. As will be indicated elsewhere in this paper, the effect of increasing the time of storage of the soil in the air-dry condition before treatment with naphthalene, is to increase the period of greatest activity of the bacteria, and to increase the time before the maximum bacterial content is reached. In view of this the apparent discrepancy between the results of Exp. V and Exp. VIII is accounted for by the fact that the soil used for Exp. V was fresh, whilst that used for Exp. VIII was about 8 months old. Thus, in consideration of Exp. V, the coincidence of the bacterial decrease with the increase in active amoebae in Exp. VIII does not indicate that the first large reduction in numbers is due to the amoebae. Evidently naphthalene in $M/10$ doses can only suppress protozoa for a given length of time, the protozoa showing periods of marked activity after 6 or 7 days, irrespective of the numbers of bacteria present at that time. This point is discussed more fully below.

Exp. IX. The increase in bacterial numbers in the treated soil has been shown to occur while the protozoa are inactive. Nevertheless it is possible that the removal of the detrimental action of the protozoa, even if coupled with the increased availability of the organic matter of the soil induced by the process of air-drying, may not be sufficient to account for the large increase in the numbers of bacteria. Attention was accordingly directed towards the hypothesis that the naphthalene could act as a source of energy for the bacteria, and therefore the effect of varying the dose was investigated. Two parallel series of five flasks each were set up, one receiving an $M/5$ and the other an $M/20$ dose of naphthalene. This experiment was carried out immediately after Exp. III, and on the same soil, so the numbers of bacteria produced by an $M/10$ dose in that experiment are given for comparison. The results are shown in Fig. 7.

It will be seen that both the $M/5$ and $M/20$ doses have given rise to smaller numbers of bacteria than the $M/10$ dose. It follows, therefore, that the increase in numbers of bacteria cannot be due solely to the enhancement of those organisms which possess the power of utilising the naphthalene as a source of energy, for if that were the case, obviously the numbers should vary directly with the amount of naphthalene present. It is true that the bacteria which decompose naphthalene must obtain their nitrogen from that available in the soil, and that consequently the amount of this is a factor which will limit the development of bacteria

capable of decomposing naphthalene. If this were the only factor limiting the numbers of bacteria, the $M/5$ dose, contrary to the results of the experiment, should have produced numbers at least as large as those produced by the $M/10$ dose. The observed numbers represent a balance of numbers of two groups of bacteria whose developments are inversely affected by the presence of naphthalene. The higher counts given by the $M/5$ dose on the fourth and fifth days are accounted for by persistence of the naphthalene and the consequent prolonged enhancement of the development of the naphthalene-decomposing group. The existence of an optimum dose is deemed to be strong evidence of the mixed nature of the population, the optimum dose being that at which the number of bacteria using the naphthalene as a source of food, plus the number developing in spite of the presence of naphthalene, is greatest.

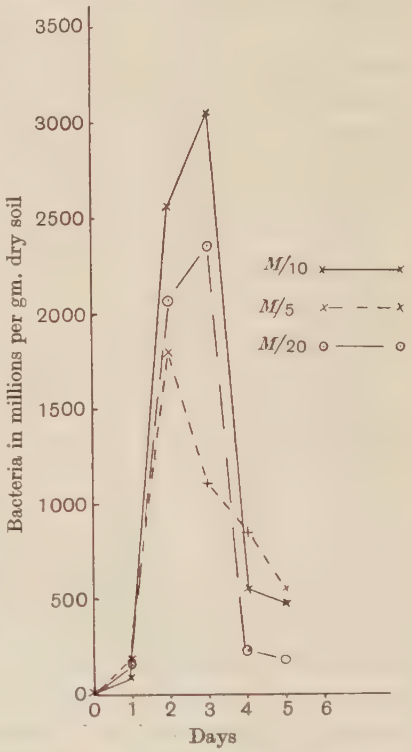

Fig. 7. Bacteria in cucumber soil treated with $M/10$, $M/5$ and $M/20$ doses of naphthalene.

Exp. X. An attempt was, therefore, made in this experiment to determine the proportion of "naphthalene-bacteria" in the total population by making counts from the same sample on Thornton's medium and also on the naphthalene medium designed specially to favour the naphthalene-decomposing group. Fig. 8 shows the results of this experiment. The curve for the naphthalene-decomposing bacteria given in Fig. 8 shows that there is a maximum development of these bacteria after 5 days has elapsed, but since no estimations of naphthalene were made this maximum cannot be correlated with the disappearance of the naphthalene from the soil. Also, these naphthalene-decomposing bacteria seem to take a permanent place in the bacterial population, even after all odour of naphthalene has gone from the soil, since the curve for the naphthalene-decomposing bacteria follows the same course after the maximum

has been reached as the curve for the bacteria developing on Thornton's medium. The average value for the percentage of naphthalene-decomposing bacteria after the maximum had been reached was 53 per cent.

Before the maximum number of naphthalene-decomposing bacteria has been attained, there is a period during which the numbers of bacteria developing on Thornton's medium are very high, whilst the numbers of bacteria on the naphthalene medium are quite low. This indicates that there is, preceding the development of naphthalene-decomposing bacteria, an increase in other soil types whose development has been favoured by

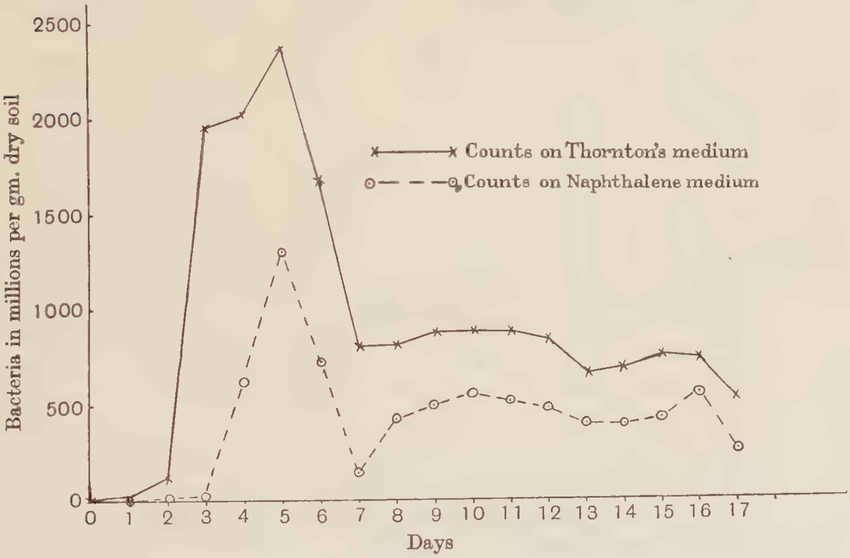

Fig. 8. Bacteria in cucumber soil treated with an $M/10$ dose of naphthalene, counted on Thornton's medium and on naphthalene medium.

the large supply of readily available organic matter in these air-dried soils. Thus the hypothesis formed as a result of Exp. IX is supported by the results from Exp. X. Other similar experiments on the same sample of soil are not recorded here, but have given comparable results.

Exp. XI. If the naphthalene were acting as a source of food for the bacteria, it should be possible to increase the numbers of naphthalene-decomposing bacteria by adding successive doses of naphthalene. In the following experiment three series of flasks were set up. Series A, B and C each received an initial dose of naphthalene; Series B and C received a second dose as soon as the first had practically disappeared; Series C

received a third dose when the second had partially disappeared. The results are given in Table VII.

Table VII.

Numbers of bacteria in cucumber soil treated with successive M/10 *doses of naphthalene.*

	Bacteria in millions per gm. of dry soil					
	Series A. 1 dose		Series B. 2 doses		Series C. 3 doses	
Days	Thornton's medium	Naphthalene medium	Thornton's medium	Naphthalene medium	Thornton's medium	Naphthalene medium
0	1	—	1	—	1	—
1	51	8	47	12	47	5
2	149	20	158	38	152	21
3	1216	93	1216	611	1664	1136
4	1920	390	1888	374	1984	1034
5	2656	1440	2464	1568	2432	2048
	Stirred, but no more naphthalene added		1 dose of naphthalene added		1 dose of naphthalene added	
6	3296	1590	2336	1056	2240	1216
7	2086	1184	1792	1504	1952	1888
8	1245	499	2400	1664	2458	2330
	Stirred, but no naphthalene added		Stirred, but no more naphthalene added		1 dose of naphthalene added	
9	1715	691	2074	1436	2144	2144
10	1488	979	2150	1533	2432	2400
11	1462	1125	2112	1765	2304	1824
12	1450	966	2470	1565	2368	1888

It will be seen that the numbers of bacteria counted on Thornton's medium are almost identical in the three series up to the point at which Series B and C received their second dose. After this, Series A shows a further increase, while Series B and C show a decrease. Whether this further increase in Series A is due to the effect of stirring the soil, or whether it is merely a case of the maximum not having been reached, is uncertain. The depression in numbers in Series B and C caused by the addition of the second dose of naphthalene is in accordance with the theory that the bacterial population produced by the first dose does not consist wholly of naphthalene-decomposing bacteria. Addition of a second dose of naphthalene might, from the experience of Matthews (1923), be expected to cause a decrease in the numbers of bacteria which do not decompose naphthalene, the depression in numbers shown by Series B and C being evidently due to this cause. Matthews (1923) showed that addition of naphthalene to fresh, undried cucumber soil (containing of course many species of bacteria), causes a depression in numbers before the increase appears. The addition of a third dose to

Series C caused no further depression in numbers, but since crystals of naphthalene were visible in the soil when the third dose was added, it seems almost certain that the bacteria which do not decompose naphthalene were still so few in numbers that the third dose could not result in any further depression. Series A shows a second increase in numbers on the ninth day, possibly due to the effect of stirring on the eighth day. The numbers of bacteria in Series B and C are maintained at a higher level from the seventh day onwards than those in Series A, this indicating that the added naphthalene was acting as a source of food for the bacteria. Consideration of the counts on the naphthalene medium shows that there is in Series A and B a period of 4 days from the start during which the numbers of naphthalene-decomposing bacteria do not show any great increase, whilst the numbers on Thornton's medium are increasing rapidly. This result is similar to that obtained in Exp. X. In Series C, on the other hand, the naphthalene-decomposing bacteria commenced to multiply rapidly after only 2 days, and indeed the numbers of these bacteria in this series were throughout much higher than in the other series. An explanation of this difference may perhaps be sought in the fact that it was not possible to accommodate all of the thirty-six flasks in this experiment on the same shelf of the incubator, and those of Series C found place on a lower shelf, where, owing to the proximity of the heating coils in the base, the temperature was probably a little higher than at the level of the other shelf. The results of Series C will therefore not be considered. The numbers of naphthalene-decomposing bacteria in Series B are definitely higher, after the addition of the second dose, than those in Series A, to which no second dose was added, this helping to show that naphthalene can act as a source of energy for bacteria. It must be admitted, however, that no explanation of the apparent increase in naphthalene-decomposing bacteria in Series A from the ninth day onwards can be found, since there was at that time no odour whatever of naphthalene in the soil. The second dose of naphthalene added to Series B after the fifth count had been made did not disappear as rapidly as the first, there being still visible evidence of it in Series B on the twelfth day. This was probably due, as previously mentioned, to the coarser nature of the powder used for the second dose. The result is, however, at variance with the finding of Tattersfield (1927) that a second dose disappears more rapidly than the first. The discrepancy is no doubt accounted for by the fact that the dosage employed by Tattersfield was very small compared with that given here, so that the slower rate of decomposition of the second dose in this investigation may be attributed

to the greater retarding effect of the accumulation of products of metabolism resulting from the large doses employed.

Exp. XII. The soil used for this experiment was from a heap of cucumber "sick-soil" which had been standing for 2 years, since its removal from the forcing house, and had become covered with a growth of weeds. The optimum water content of this soil was 40 c.c. per 100 gm. of dry soil. Two series, A and B, were prepared. Both series received an initial treatment with naphthalene, whilst Series B received an additional dose after 3 days. The results are given in Table VIII.

Table VIII.

Numbers of bacteria in cucumber soil treated with successive M/10 *doses of naphthalene.*

	Bacteria in millions per gm. of dry soil			
	Series A. 1 dose		Series B. 2 doses	
Days	Thornton's medium	Naphthalene medium	Thornton's medium	Naphthalene medium
0	18	5	18	5
1	256	46	(256)	(46)
2	3955	119	3465	91
3	7035	1575	8295	455
	Stirred, but no more naphthalene added		1 dose of naphthalene added	
4	5495	665	1015	245
5	3570	770	315	245
6	1715	1155	525	175
7	1015	490	1225	455
8	945	210	700	119

It is evident at once that this sample of soil was much richer in easily decomposable organic matter than the preceding ones. The maximum numbers of bacteria developing on Thornton's medium are more than twice as large as any previously recorded, and since the counts made on the naphthalene medium are not correspondingly higher, it follows that the higher numbers must be due to a greater multiplication of bacteria which do not decompose naphthalene. That this is the case is borne out by the enormous depression in numbers produced by the addition of the second dose of naphthalene. The results of the counts on the naphthalene medium neither agree with the results of previous experiments nor do they agree amongst themselves. The numbers of naphthalene-decomposing bacteria are definitely lower in Series B after the addition of the second dose than they are in Series A—a result in direct opposition to that previously obtained. Also the counts on the naphthalene medium in Series A and B on the third day, which should agree fairly closely, are

widely different. In view of these conflicting results no definite conclusions can be drawn from the counts on the naphthalene medium. Possible causes of these conflicting results are discussed later. In view of the anomalous result given by this experiment it was repeated, using a freshly-dried sample of a soil in good condition. Results similar to those of Exp. XI were obtained.

Exp. XIII. The sample of soil used in Exp. XII having been standing in a heap for 2 years might have become deficient in lime, and the enormous depression in numbers produced by the second treatment with naphthalene might have resulted from the exhaustion of the lime reserve consequent upon the previous excessive bacterial activity. The lime reserve in this sample of soil was, therefore, estimated by shaking 10 gm. of the dry soil with excess of HCl and allowing to stand for 3 hours, the amount of acid neutralised being found by titration with $N/10$ NaOH. The result showed that the lime reserve was actually larger in the "sick-soil" than in the soil previously used, being equivalent to 103 c.c. $N/10$ NaOH in the "sick-soil" and 75 c.c. in the other. It was, therefore, considered unlikely that lack of lime was the cause of the rapid fall in the numbers of bacteria.

Exps. XIV and XV. In these experiments the bacteria have been counted by the new direct method of Gray and Thornton (1928, *b*), estimations of naphthalene have been made, and the *p*H of the soil has been measured daily. In addition (in Exp. XV) estimations of ammonia were included; these are considered separately under the heading of Exp. XVI. Two series of flasks, A and B, have been set up in each experiment, both series receiving an initial treatment with naphthalene. In Series B a second addition was made after 3 days, and after a further period of 4 days the remaining flasks were divided into two sets, one of which, Series C, received a third quantity of naphthalene. The results of one of these experiments (Exp. XIV) are shown diagrammatically in Figs. 9 and 10, the results of the other being omitted as they were essentially similar. The sample of "sick-soil" was used for these experiments.

It will be seen (Fig. 9) that the bacterial numbers in Series A continued to rise after the naphthalene had practically all disappeared, and this may be either a result of stirring the soil, or of the utilisation by the bacteria of some decomposition product of the naphthalene. It will be noted that stirring the soil does not always result in higher numbers; for instance, in this experiment stirring on the seventh day led to a decrease in numbers, and a like result was also obtained on the fourth day in

Exp. XII. The addition of a second dose of naphthalene to Series B has led to an increase in bacterial numbers, when these are obtained by the direct method, whereas when bacteria are counted by the plating method a temporary slight decrease is observed (see Table VII). The discrepancy

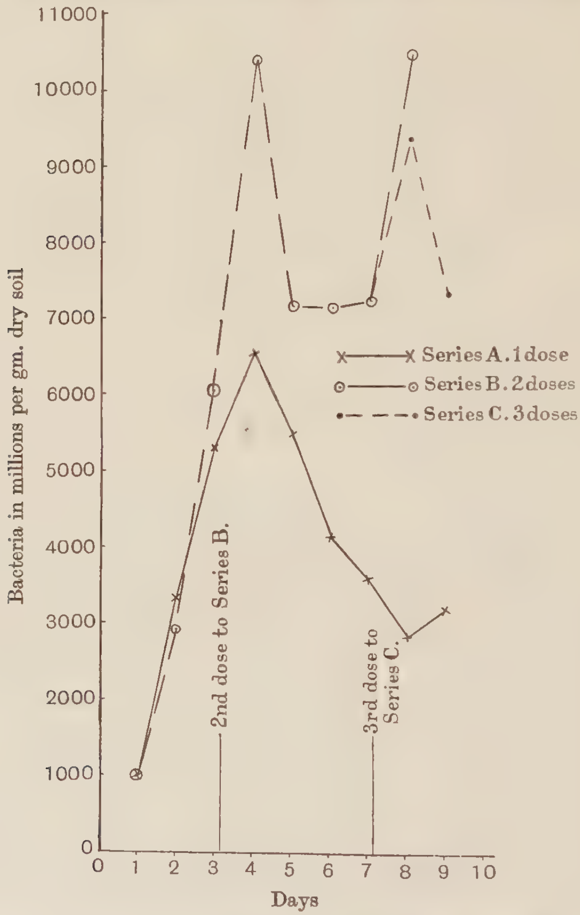

Fig. 9. Bacteria in cucumber soil treated with successive $M/10$ doses of naphthalene, counted by a direct method.

is evidently to be accounted for by the fact that the direct method will include bacteria which have just died as a result of the addition of the second dose of naphthalene. The counts obtained by the direct method after the addition of the second dose of naphthalene, therefore, represent the whole increase in numbers of bacteria produced by the first dose plus

any increase in numbers of certain species consequent upon the addition of the second dose; the counts obtained by the plating method represent the numbers surviving the second addition of naphthalene plus any increase in numbers of certain species produced by the second dose. The increase in numbers of bacteria in Series B, following the addition of the second dose of naphthalene, was much greater than that in Series A, to which no second dose was added. It is, therefore, concluded that this increase was due to the multiplication of naphthalene-decomposing organisms. The fall to a lower level of the numbers of bacteria in Series B

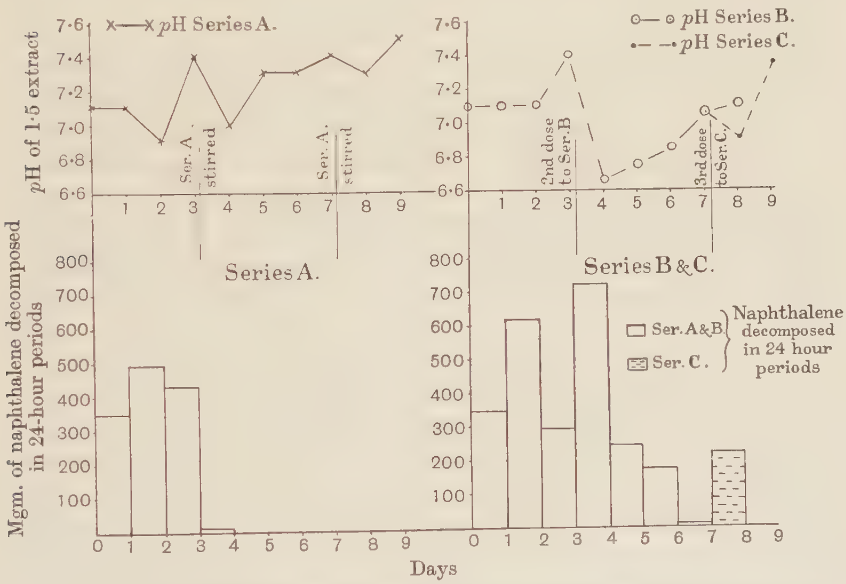

Fig. 10. *p*H and naphthalene decomposition in cucumber soil treated with successive *M*/10 doses of naphthalene.

which occurs between the first and second days after the addition of the second dose may be attributed to the disappearance of the effects of stirring. In conformity with the previous results obtained by the plating method of counting, the numbers of bacteria in Series B remain higher than in Series A. Stirring Series B on the seventh day results in increased bacterial activity, but does not lead to the disappearance of any more naphthalene. The addition of a third dose of naphthalene in Exp. XIV did not result in any increase in bacterial numbers which could not be accounted for by the effects of stirring, as will be seen on comparing the curves for Series B and C in Fig. 9. A quantity of naphthalene seemed

to have disappeared at this point, however, but this quantity may not have been significant. On the other hand, in Exp. XV (results not shown) the addition of the third dose resulted in a greater increase in numbers of bacteria than could be accounted for by stirring effects, while a larger quantity of naphthalene, in this case deemed to be significant, had disappeared.

The results of the naphthalene estimations (Fig. 10) show that the rate of disappearance of the first dose increased steadily. The smaller amount of naphthalene decomposed between the second and third days does not indicate a slower rate of disappearance, since the amount decomposed represents practically all the naphthalene available during that period. The second dose was attacked with much greater energy than the first, 50–60 per cent. of it being decomposed within 24 hours of its addition. The rate of decomposition then slowed down rapidly, till a point was reached at which no more naphthalene was being decomposed. The addition of a third dose led to the disappearance of a further small quantity, but decomposition ceased again within 48 hours. These results are fully in keeping with bacterial destruction of the naphthalene. Multiplication of the naphthalene-decomposing organisms would naturally result in an increasing rate of disappearance of the naphthalene, which rate would reach a maximum and subsequently fall off in proportion to the accumulation of the products of metabolism.

It would seem from a consideration of the pH determinations (Fig. 10) that during the disappearance of the first dose of naphthalene the pH first remained approximately steady and then rose. The addition of a second dose caused the pH to fall very markedly in Exp. XIV, but not so markedly in Exp. XV. The pH then tended to rise slowly. The addition of a third dose seemed to have a very slight depressant action on the pH, but this effect was uncertain. The irregularity of the curves is probably due to the inaccuracies in the technique employed, but on the whole it would seem that one dose of naphthalene results in the soil becoming more alkaline than the control, whereas the addition of a second dose results in a temporary acidity, which is probably due to carbon dioxide produced by oxidation of the naphthalene.

Exp. XVI. The figures for the ammonia estimations are given in Table IX. It will be seen that drying the soil resulted in an accumulation of ammonia to the extent of 110 parts per million. During the period in which the first dose of naphthalene was being decomposed, this store of ammonia disappeared, and was evidently utilised as a source of nitrogen by the naphthalene-decomposing bacteria.

Table IX.

Ammonia content of cucumber soil treated with successive M/10 *doses of naphthalene.*

	Ammonia in parts per million of dry soil			
Days	A	B	C	Control
0	110	110	—	110
1	75	75	—	110
2	10	8	—	100
3	29	1*	—	105
	Stirred: no naphthalene	1 dose of naphthalene		
4	63	3	—	102
5	163	6	—	74
6	223	4	—	49
	Stirred: no naphthalene	Stirred: no naphthalene	1 dose of naphthalene	
7	295	5	5	20
8	252	12	5	4
9	—	19	—	3

* The figures for the disappearance of naphthalene from the soil showed that a much larger quantity of naphthalene was decomposed in Series B between the second and third days than in Series A, and the lower figure for the ammonia in Series B is attributed to this cause.

When no further addition of naphthalene was made ammonia began to accumulate at once, the slight increase in ammonia on the third day in Series A showing that practically all the first dose must have disappeared very soon after the second sample was taken. The fact that there was finally a larger amount of ammonia present than at the start shows that some of the organic matter of the soil had been decomposed. The slight fall in the ammonia content on the ninth day in Series A indicates that nitrification was by that time becoming important. When a second dose of naphthalene was added the ammonia content became practically zero, and did not begin to rise again until naphthalene decomposition had ceased. The addition of a third dose suppressed even this slight increase. In the control the ammonia content gradually fell to zero, probably owing to nitrification. The relationship between the disappearance of naphthalene and the ammonia content of the soil affords very strong support for the theory that the naphthalene is decomposed by bacterial action. The soil from which these ammonia figures were obtained was, however, particularly rich in easily decomposable organic matter, as is shown by the very high bacterial counts obtained in Exp. XII. It is therefore highly probable that in a more normal soil the variations in ammonia content would not be so pronounced, and might be alto-

gether obscured by the action of the nitrifying organisms. Further work on this subject, together with a study of the variations in nitrate content is evidently needed, and will shortly be undertaken.

Exp. XVII. Thirteen strains of bacteria have been isolated from the naphthalene medium plates, and all of these make as good growth on this medium, which contains no other source of carbon than naphthalene, as they do on the ordinary media. These strains have not yet been fully characterised, so it is impossible to state whether they are identical with the organisms described by Tausson (1927), or Gray and Thornton (1928, *a*). When grown on naphthalene-agar slants they all produce a yellowish brown pigment which gradually darkens in colour. Meanwhile, in all cases except one, the surface growth assumes a dark brown colour and ultimately resembles a tarry mass. At the same time the medium becomes strongly acid. It was thought possible that this acidity might be due either to the production of phthalic acid by partial oxidation of the naphthalene, or to the liberation of sulphuric acid from the ammonium sulphate in the medium as a result of the utilisation of the ammonia by the bacteria. Therefore one of the strains of naphthalene-decomposing bacteria was cultivated in mineral salt solutions, in which the nitrogen was supplied in the form of (*a*) 1 per cent. $(NH_4)_2SO_4$, (*b*) 1 per cent. KNO_3, (*c*) 0·5 per cent. $(NH_4)_2SO_4$ plus 0·5 per cent. KNO_3. Carbon was supplied in the form of naphthalene only (1 per cent.). The cultures were made in 300 c.c. conical flasks each containing 100 c.c. of medium, and all of them were inoculated with 1 c.c. from the same suspension of the organism in distilled water. The reactions of the solutions were measured after 1, 2, 3 and 6 days' growth at room temperature (12–15° C.). The results, given in Table X, show conclusively that only the cultures containing ammonium sulphate became acid. The most rapid development occurred in the ammonium sulphate cultures, but ultimately the culture containing potassium nitrate showed the best growth. Moreover, the fact that the culture containing both potassium nitrate and ammonium sulphate showed as great an acid production as that containing ammonium sulphate alone, indicates that the organism prefers ammonium salts to nitrates as a source of nitrogen. Nitrite was not produced in the culture containing nitrate. Gas was evolved in all cultures, and proved to be carbon dioxide, which is therefore one of the ultimate products of the bacterial decomposition of naphthalene.

The cultures were tested for the presence of phthalic acid in the following way. A little resorcinol was added to 2 c.c. of the culture fluid in a test-tube and the water boiled off. The residue was then kept in a

molten state until it had assumed a dark red colour, when it was allowed to cool, dissolved in water, and a few drops of caustic soda added. The presence of phthalic acid is indicated by the production of fluorescein. The test is capable of giving a positive result with only 0·1 mgm. of potassium hydrogen phthalate in 1 c.c. of water, the conditions essential for success being that all the water must be boiled off, and the residue heated till it is dark red. All cultures were proved to contain phthalic acid in small quantities after 15 days' growth, the largest amount being present in the culture containing potassium nitrate. Tests on the control flasks gave negative results. Phthalic acid is therefore an intermediate product in the decomposition of naphthalene by this organism. In order to verify this the organism was inoculated into mineral salt solutions containing phthalic acid as the sole source of carbon. After 3 days at room temperature the cultures were turbid. The bacteria isolated by Tausson (1927) did not produce phthalic acid and did not grow in a phthalic acid medium. He therefore concluded that the bacterial decomposition of naphthalene involved a simultaneous attack on both rings of the naphthalene molecule.

Table X.

The growth of a naphthalene-decomposing organism in the presence of varying forms of nitrogen supply.

Nitrogen supply	pH of solution Days after inoculation					Growth in solution Days after inoculation		
	0	1	2	3	6	2	3	6
Ammonium sulphate	6·9	6·8	6·65	6·45	4·4	Turbid	Turbid	Clearing
Uninoculated control	6·9	6·9	—	—	6·9	Sterile	Sterile	Sterile
Potassium nitrate	7·05	7·05	6·95	6·95	7·5	Very faintly turbid	Definitely turbid	Very heavy growth
Uninoculated control	7·05	7·05	—	—	7·0	Sterile	Sterile	Sterile
$(NH_4)_2SO_4$ plus KNO_3	7·0	6·8	6·7	6·6	4·4	Turbid	Turbid	Clearing
Uninoculated control	7·0	7·0	—	—	6·95	Sterile	Sterile	Sterile

Exp. XVIII. Under the experimental conditions of this investigation it has been found that naphthalene decomposition and bacterial multiplication began at once, there being no initial lag such as is described by Matthews (1923) and Tattersfield (1927). These latter workers have however used fresh undried soils. Accordingly an experiment was performed in order to determine the relative rates of disappearance of

naphthalene in dried and undried soils, using a dose of naphthalene of 50 mgm. per 100 gm. of soil (on the dry soil basis), the temperature of incubation being 20° C.

The results, given in Table XI, show clearly that fresh, undried soil decomposes naphthalene much more rapidly than dried soil, so that the absence of an initial lag in the decomposition cannot be ascribed to the use of air-dried soil. The greater activity of fresh soil is most probably due to the larger population of bacteria.

Table XI.

The decomposition of naphthalene in dried and undried soil at 20° C.

Naphthalene added (mgm.)	Time of incubation (hours)	Naphthalene decomposed (mgm.)	
		Dried soil	Undried soil
50	22	15·1	50
50	24	20·1	49·1

Exp. XIX. Attention was then directed towards the temperature of incubation of the treated soil, since the workers cited above have used air-temperature for their experiments. Cucumber soil was therefore treated with naphthalene at the rate of 1280 mgm. per 100 gm. of dry soil, and comparison made of the disappearance of naphthalene at temperatures of 20° C. and 16° C. The results are given in Table XII.

Table XII.

The influence of temperature upon the disappearance of naphthalene from cucumber soil.

Naphthalene added (mgm.)	Time of incubation (hours)	Naphthalene decomposed (mgm.)	
		20°	16°
1280	24	472	12
1280	48	950	564
1280	72	1270	1258

It is evident that at the lower temperature an initial lag in the naphthalene decomposition makes its appearance. It was shown experimentally that this result was not a fictitious one due to more rapid volatilisation of the chemical at the higher temperature. When sterilised soil was treated with naphthalene and incubated at 20° C. it was found that only a very small fraction of the naphthalene added had disappeared after 1, 2 and 3 days.

The temperature of incubation of the soil, therefore, appears to determine whether an initial lag period in the naphthalene decomposition

will or will not be observed. Evidently at the higher temperature the activity of the naphthalene-decomposing organisms is greatly enhanced, and it appears probable that at that temperature the partial sterilisation effect observed by Matthews (1923) would be entirely masked by the rapid development of these organisms.

Discussion.

The addition of naphthalene to cucumber soil causes a large increase in the numbers of bacteria, as has been shown previously by Matthews (1923) and Tattersfield (1927). These investigators have, however, stated that there is a preliminary period of from 2 to 3 days before either the numbers of bacteria begin to increase or the naphthalene begins to disappear, and they attributed this to a possible reaction of the naphthalene with some substance in the soil preceding its decomposition by the bacteria. In this investigation, provided that the soil had not been in the air-dry condition for more than about 8 weeks, no preliminary period has been observed, the bacteria beginning to multiply rapidly and the naphthalene to disappear at once. (The preliminary period which makes its appearance when soils that have been stored for a lengthy time in the air-dry condition are used is deemed to be of a different nature to that observed by Matthews (1923) and Tattersfield (1927); the cause of its appearance is discussed later.) It was considered that a possible explanation of these different results might lie in the fact that the soils used by the above investigators were fresh undried soils, and as such contained a very small amount of ammonia, while in the experiments described above air-dried soils containing a large amount of ammonia have been employed (see Exp. XVI). It has been shown that a strain of naphthalene-decomposing bacteria develops much more rapidly when nitrogen is supplied in the form of ammonium sulphate than when it is supplied in the form of potassium nitrate (see Exp. XVII), so that in the air-dried soils the development of naphthalene-decomposing bacteria would probably be more rapid than in the fresh soils. On this assumption the preliminary period observed by Matthews (1923) and Tattersfield (1927) would be due to the slow initial growth of the naphthalene-decomposing organisms in the absence of ammonium salts. Whilst the presence of ammonium salts would have a favourable effect on the multiplication of naphthalene-decomposing bacteria, it has been shown that actually this beneficial effect is obscured by the adverse one due to the reduction in the bacterial population produced by the air-drying of the soil, the presence or absence of a lag period being dependent on the

temperature of incubation of the treated soil. The lag period is, therefore, due to the slow rate of multiplication of the naphthalene-decomposing bacteria at air-temperature.

It is interesting to note that the length of time during which the soil has been in the air-dry state has a marked influence upon the fluctuations in numbers of bacteria produced by the addition of naphthalene to the soil. As the time of storage is lengthened a preliminary period, during which the numbers of bacteria increase slowly, makes its appearance. Also, the maximum number of bacteria obtainable by the addition of an $M/10$ dose becomes gradually less, whilst the length of time intervening between the rapid rise and fall in numbers increases. These effects are brought out clearly in Table XIII, and are in all probability due to a progressive reduction in the numbers and the vigour of the bacteria in the air-dried soils, and also to the physical changes induced by the process of air-drying.

Table XIII.

The effect of the time of storage of the air-dry soil on the bacterial increase produced by an M/10 *dose of naphthalene.*

Time of storage of air-dried soil (days)	Period before rapid multiplication of bacteria begins (days)	Maximum numbers of bacteria in millions	Period between rapid rise and fall in numbers (days)
20	0	3200	2
70	1	3038	2
240	2	2384	3
280	2	3296*	4
370	2	1920*	5

* These counts have been subject to the enhancing effect of stirring the soil.

Buddin (1914, *b*) has observed an increase in the available nitrogen in field soils which, after having been air-dried, have been re-moistened and incubated, this increase being unaccompanied by a corresponding increase in bacterial numbers. Waksman and Starkey (1923) state that air-drying of field soils followed by re-moistening to the optimum water content results in a short period during which the activity of micro-organisms is stimulated, while nitrates temporarily decrease in amount. Lebedjantzev (1924) has recorded an increase in fertility of field soils as a result of air-drying. The present work has shown that the effect of air-drying cucumber soils is to increase the ammonia content, whilst during subsequent incubation of the re-moistened soil, the accumulated ammonia gradually disappears (Exp. XVI). Also a slight increase in the bacterial numbers obtained by the plate count is observed.

Matthews (1923) concluded that the increase in bacterial numbers obtained by the addition of naphthalene to cucumber soils was due to nutrition of the bacteria by the naphthalene. Whilst this may be the case when fresh soils are used, evidence has been obtained to show that, when the soil has been air-dried before use, only a part of the bacterial increase is due to naphthalene-decomposing bacteria. Matthews (1923) has shown that when corked bottles are used the bacterial numbers obtained increase as the amount of naphthalene added is increased from $M/100$ to $M/50$ and $M/10$. The present investigation has shown that when cotton-wool stoppers are employed, $M/10$ is the optimum dose at 20° C., as a further increase in the amount of naphthalene to $M/5$ reduces the numbers obtained. Matthews, using cotton-wool stoppers, finds that $M/50$ is the optimum dose at 18° C.

An accumulation of ammonia has been observed as a result of the addition of naphthalene to cucumber soil, although Matthews (1923) stated that there was no such accumulation. This latter statement has been based upon ammonia estimations made at intervals of 7, 24 and 77 days from the start and, in view of the rapidity with which changes take place in these rich soils, it is suggested that a temporary increase in ammonia may have been overlooked. Skinner (1926) strongly criticised the view that the bacterial increase obtained by the addition of non-nitrogenous antiseptics to the soil was a result of the decomposition of the antiseptics by bacteria, on the ground that such decomposition would result in a decrease and not in an increase in available nitrogen. The present investigation has shown that in the case of naphthalene there is, in a certain abnormally rich soil, a decrease in ammonia, but only during the period in which naphthalene is disappearing from the soil. Immediately naphthalene decomposition ceases ammonia begins to accumulate. Evidently in the case of air-dried soils the naphthalene-decomposing bacteria obtain their nitrogen, firstly from the ammonia produced by the process of air-drying, and subsequently from some other source, such as nitrate or nitrogenous organic matter. Directly the supply of naphthalene is exhausted, the bacteria commence their attack upon the organic matter of the soil, including the bodies of dead bacteria and protozoa, and the large bacterial population soon produces an accumulation of ammonia. The slight fall in the ammonia content on the eighth day in Exp. XVI indicates that nitrification was beginning to make its influence felt. Probably therefore naphthalene has no detrimental effect on the nitrifying organisms, so that the accumulated ammonia would shortly have disappeared. Matthews (1923) has obtained evidence to

show that at 18° C. naphthalene has no effect on the nitrifying organisms. A further study of the ammonia and nitrate relationships in treated soils is about to be undertaken.

It has been shown that naphthalene, when added to air-dry soil, temporarily arrests the development of protozoa, and this effect must be one of the factors tending towards the enhancement of the bacterial numbers. The increase in numbers of bacteria produced by a second dose of naphthalene, added some days after the first had disappeared (see Exps. I and III), is not so large as that produced by the first dose. This is probably due to the accumulation of the decomposition products resulting from the first treatment with naphthalene. There may also be a set-back to the multiplication of bacteria produced by the addition of a second dose of naphthalene (see Exp. III). Since such interruption in the bacterial increase is seen in both the dried and the undried series, it would seem that in soils in which there has recently been a large amount of activity among both bacteria and protozoa, the effect of a second dose of naphthalene on the protozoa is very slight, and is comparable with that of a short period of air-drying.

It has been shown that in cucumber soil treated with one dose of naphthalene there are periods during which the numbers of active amoebae increase very rapidly. During the intervals between these periods there are apparently no active amoebae, but this is probably a fictitious result, since, as has been pointed out previously, the method of counting protozoa is not sufficiently accurate to detect small variations in large numbers of protozoa such as have been encountered in this investigation. The periods of activity occur at irregular intervals, and are of very short duration, but each period coincides with a decrease in the numbers of bacteria. Cutler and Crump (1927) have shown that when a species of soil amoeba, *Hartmanella hyalina*, was grown in pure culture with only one species of bacteria as food, its rate of reproduction increased as the ratio of bacteria to amoebae rose. A similar condition has been shown to hold in the case of the ciliate *Colpidium colpoda* (Cutler and Crump, 1924). In order to determine how far in this investigation the rate of reproduction of the amoebae has depended on the supply of bacterial food, the rates of reproduction of the amoebae, and the ratios of bacteria to amoebae over 24-hour periods, have been calculated from the results of Exp. V, and are given in Table XIV. The rates of reproduction of the amoebae have been calculated on the usual logarithmic basis. The bacterial ratios have been calculated by dividing the average number of bacteria per gm. of wet soil in each 24-hour period by the

average number of active amoebae present during the same period. Cyst formation, being very slight in this experiment, will not seriously affect the calculations.

Table XIV.

The relation between the rate of reproduction of amoebae and the supply of bacterial food.

Period	Ratio of bacteria to active amoebae	Reproduction rates of amoebae
1	400,000 : 1	3·7
2	150,000 : 1	1·0
3	110,000 : 1	0
4	16,000 : 1	3·3
5	7,500 : 1	0
6	20,000 : 1	Negative

It would seem that the bacterial ratio governs the rate of reproduction of the amoebae for the first three 24-hour periods, after which there is a sudden increase in the rate which cannot be accounted for by a corresponding increase in the bacterial ratio. However, this high rate of reproduction ceases almost immediately, probably owing to the bacterial ratio being too low. It would appear, therefore, that the sudden increases in the numbers of active amoebae are not connected with the available food supply, but are due to some other factor, which may perhaps be connected with the life cycle of the amoeba. The sudden cessation of this multiplication may be due to lack of food. It must not be forgotten, however, that in the above calculations no account has been taken of the numbers of bacteria which are unable to develop on Thornton's medium, or of the fact, proved by Cutler and Crump (1927), that all bacteria are not of equal value as food material for protozoa.

It has been pointed out previously that conflicting results have been obtained from the experiments in which counts have been made on the naphthalene medium. In view of the fact that it is impossible to ensure that each tube of the medium shall contain exactly the same quantity of naphthalene in the same state of division, irregularities in the results are only to be expected. It is not, however, clear why counts made on different samples of freshly air-dried soils should give results in direct opposition to one another. The soil which gave lower numbers of naphthalene-decomposing bacteria with two doses of naphthalene than with one dose was a "sick-soil," and it is suggested that the commencement of bacterial activity consequent upon the addition of the second dose, following on the abnormally large activity already produced by the first dose, may have resulted in an accumulation of toxic products to such an

extent that all species of bacteria, including the naphthalene-decomposing bacteria, were reduced in numbers.

The bacterial counts obtained by the direct method will naturally be higher than those given by the plating method, since organisms which do not grow on nutrient media will be included. Comparison of the curves obtained by the plating and by the direct methods of counting bacteria shows that the fall in numbers is much more rapid by the plating method than by the direct method. Examination of the slides made for the purposes of the direct count has shown that, for the first 3 or 4 days, the majority of the bacteria are isolated from one another, but that after the fourth day clumps of bacteria become much more numerous, and isolated bacteria relatively few in numbers. The formation of clumps will result in lower counts by the plating method and, therefore, this is one of the causes of the greater rapidity of the fall in numbers shown when counts are made by this method. Moreover, the direct method will include bacteria which have only recently died. Although these organisms will not be viable on nutrient media, yet their enzymes are presumably still active, so that for the purpose of estimating the bacterial activity in the soil, such organisms ought to be included in the numbers of "living" bacteria. In this connection, it may be remarked that Quastel and Wooldridge (1926) have shown that the cells of *B. coli*, after having been rendered non-viable with toluene, are still capable of "activating" certain substances, thus causing them to reduce methylene blue.

When repeated doses of naphthalene are added to the soil, the slides show that isolated organisms form the greater part of the numbers for a much longer period than when only one dose is added. During the period of naphthalene decomposition, the direct method of counting enables one to observe that the bacterial flora is mainly of one morphological type, namely short plump rods with rounded ends. As soon as naphthalene decomposition has ceased, the bacterial flora changes in type, cocci and long rods making their appearance. These observations thus tend to confirm the theory that naphthalene is decomposed by specific bacteria in the soil.

Summary.

1. The effects of treating cucumber soil, previously air-dried, with naphthalene, are as follows: (*a*) The number of bacteria immediately increases rapidly, but very soon decreases again, rapidly at first, and then more slowly. (*b*) The development of protozoa is suppressed until most of the naphthalene has disappeared, after which rapid multiplication

begins. (*c*) Ammonia (in an abnormally rich soil) first decreases in amount, and then accumulates to a considerably greater extent.

2. The increased bacterial population is composed partly of organisms which are using the naphthalene as a source of food, and partly of organisms which do not decompose naphthalene.

3. The history of the protozoa in the soil after the disappearance of the naphthalene is one of bursts of activity at irregular intervals. Each period of activity of the amoebae results in a decrease in the numbers of bacteria. The periods of activity of the flagellates are not closely related to fluctuations in the bacterial numbers.

4. The naphthalene-decomposing bacteria utilise the supply of available nitrogen in the soil; as soon as the supply of naphthalene becomes exhausted, these bacteria attack the organic matter of the soil, resulting in an increase in the amount of ammonia in the particular soil under investigation.

5. The presence or absence of an initial lag in the naphthalene decomposition depends upon the temperature of incubation of the treated soil.

6. Strains of bacteria capable of using naphthalene as their sole source of carbon have been isolated. One of these strains has been shown to produce phthalic acid as an intermediate product in the decomposition of the naphthalene.

7. The reaction of the soil tends to become more alkaline after the disappearance of a quantity of naphthalene. Treatment with a second dose results in a temporary production of acid, which is probably carbonic acid.

ACKNOWLEDGMENTS.

The author wishes to thank Sir John Russell, Director of the Rothamsted Experimental Station, for permission to work in the laboratories there, and Mr D. Ward Cutler, Head of the Department of General Microbiology at Rothamsted, for his help and instruction in soil protozoology. Finally, the author wishes to express his deep appreciation of, and gratitude for, the useful advice and constructive criticism given so generously throughout by Prof. S. G. Paine, under whose direction the work was carried out.

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(*Received February* 18*th*, 1930.)

REVIEW

Laboratory and Field Ecology. By VICTOR E. SHELFORD. Pp. xii × 608. 219 figs. in text. London: Baillière, Tindall and Cox, 1929. 45*s*. net.

The sub-title of this book is "The Responses of Animals as Indicators of Correct Working methods" but, although it is thus addressed primarily to the zoologist, its contents are so promiscuous that all who are interested in general biology will find it a worth-while volume. To the plant ecologist it will be almost as valuable as to the animal ecologist, whilst the more strictly laboratory experimentalist will find it of great practical help. It is, in fact, a book to be in the hands of anyone interested in the experimental study of the distribution, behaviour, and general physiology of animals and plants and not a work merely for the ecologist. One may perhaps remark that the author's views as to the nature and scope of ecology, as such, while novel and stimulating, are unlikely at present to command general acceptance.

After a preface and general introduction the chapters deal with the following subjects: I. Problems of ecology and climatology; II. Methods and results of biotic observation and experimentation; III. Behaviour and acclimation; IV. Food and food effects; V. Soil and growth of food plants; VI. Dormancy and other quiescent states; VII. Animals in relation to temperature; VIII. Control and measurement of temperature; IX. Ventilation under experimental conditions; X. Control and measurement of moisture; XI. Temperature and humidity combination; XII. The effect and measurement of air pressure and evaporation; XIII. Light conditions and effects; XIV. Measurement and control of light; XV. The evaluation of factors other than temperature and moisture, and the comparison of species; XVI. Location and planning of buildings and equipment for the simulation of climatic conditions; XVII. Physical conditions in water (except light); XVIII. Light conditions in water; XIX. Chemical conditions in water; XX. Location and planning of buildings for the simulation of aquatic conditions. There are four useful appendices dealing with: I. Records; II. Equipment for keeping animals alive; III. Estimated cost of materials and equipment; IV. Sources of equipment. A Bibliography of over 1000 titles, mostly American, is followed by a somewhat inadequate Index. The book is well illustrated by 219 text-figures, most of which portray details of apparatus.

In Appendix III the author outlines five sets of equipment ranging from the simplest at $15 to one at $2500, and notes that "a small plant built on the principles discussed in Chapter XVI probably can be built for $300,000 or less." One might just as well hitch one's wagon to a star.

Regarded as a compendium of apparatus and methods for experimentation on animals and plants living in a controlled environment the book is probably unequalled; certainly I know of no other containing so much helpful material under one cover. The only criticisms one would make of these pages are that they are often written in an unnecessarily difficult and technical manner and that it is not always easy

to distinguish between apparatus which Prof. Shelford found successful and that with which he failed; for both are described.

The size and comprehensiveness of the experiments described by the author reflect not only a tremendous enthusiasm but also a very great expenditure of time and money and what must, at present, be regarded as unique facilities for this kind of work. For example, of out-door observations on animals in experimental containers the author writes: "Such a program of experiments requires 6000 to 10,000 individuals in each life-history stage and several men to carry on the work. The experiments should be repeated during each of two or three years, if possible."

One, perhaps obvious, note of warning might be sounded to anyone stimulated by this book to undertake similar work. It is the outcome of our practical experience at Rothamsted that where an elaborate piece of control apparatus has been gradually devised and lovingly built up by one investigator, it is by no means an easy task for even a skilled experimentalist accustomed to dealing with complicated mechanisms, to take over that apparatus as a going concern and get it to work successfully. There is much virtue in beginning with jampots and saucers and growing up with one's own apparatus.

WILLIAM B. BRIERLEY.

PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

I. Friday, *October 24th*, 1930.

(*a*) "Growth Studies on Oats." By M. A. H. Tincker, Esq.

(*b*) "The Yielding Capacity of Oat Varieties under different Conditions of Soils and Climate." By M. Jones, Esq.

(*c*) "The Differentiation of the Wheat Ear." By Dr P. S. Hudson.

II. Friday, *November 21st*, 1930.

"The Specific Resistance of Willows to Insect Attack." By Dr H. E. Barnes.

III. Friday, *December 12th*, 1930.

"The Purification of Waste Waters from Beet-Sugar Factories."

(1) "Microbiological Aspects." By Mr D. W. Cutler, M.A.

(2) "Biochemical Aspects." By Mr E. H. Richards, F.I.C.

NOTICES

IMPERIAL BOTANICAL CONFERENCE, 1930.

The following Resolution was put from the Chair and carried unanimously:

"That an Imperial Botanical Conference take place in England in 1935 shortly before the International Botanical Congress which is to be held in that year in Holland."

The following Interim-Committee was appointed: the Director of Kew (Convener); the Keeper of Botany, Natural History Museum; the Professors of Botany at Oxford and Cambridge; a Professor of Botany of the University of London (to be nominated by the Chairman of the Board of Studies of the University); one representative of the Colonial Office and one representative of the Dominion Office.

It was further resolved that this Committee summon a meeting of British botanists in the near future for the purpose of appointing an Executive Committee for the said Conference.

ERIKSSON PRIZES.

In connection with the International Conference for Phytopathology and Economic Entomology, held in Holland in 1923, prizes were offered in 1928 for the two best memoirs concerning (1) investigations on rust diseases (Uredineae) of cereals, and (2) investigations on the rôle played by insects or other invertebrates in the transmission or initiation of virus diseases in plants, the prizes being of the value of 1000 Swedish crowns (about £55) each. It is now announced that the prize for the most meritorious investigations on rust has been awarded to Mr J. H. Craigie, Senior Plant Pathologist in Charge, Dominion Rust Research Laboratory, Winnipeg, Manitoba, Canada. The adjudicators have made no award in connection with the subject for the second prize.

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D. WARD CUTLER

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STUDIES ON POTATO VIRUS DISEASES

IX. SOME FURTHER EXPERIMENTS ON THE INSECT TRANSMISSION OF POTATO LEAF-ROLL

By KENNETH M. SMITH, D.Sc., Ph.D.

(*Potato Virus Research Station, School of Agriculture, Cambridge.*)

(With Plate XI and 5 Text-figures.)

CONTENTS.

I. On a possible relationship between the virus of potato leaf-roll and its insect vector.

One of the chief points of evidence in favour of an obligate relationship between a plant virus and its insect vector is the existence of what may prove to be an incubation period of the virus within the insect before the latter is infective to a healthy plant. At present this would be more correctly termed a delay in the development of infective power in the insect. Several such waiting periods are described in studies upon plant viruses. Kunkel(6) finds that the leaf hopper *Cicadula sexnotata*, the vector of aster "yellows," does not become infective until a period of at least 10 days has elapsed since the first feeding on a "yellowed" plant. Similarly, in the case of "curly-top" of the sugar-beet, the leaf hopper *Eutettix tenellus* Baker is not infective until about 24 hours after the first contact with the source of infection(11).

The aim of the experiments now to be described was to discover

whether there exists a similar waiting period in the case of potato leaf-roll and the aphis vector *Myzus persicae*. In the two instances above quoted, the leaf hoppers appear to be specific to the respective viruses which are not transmissible by any other insect, although recent work by Fawcett (5) seems to show that, in the Argentine, curly-top of sugar-beet is transmissible by another leaf hopper, *Agallia sticticollis*. However that may be, evidence is brought forward in a later section of this paper to prove that there is no absolutely specific relationship between *M. persicae* and the leaf-roll virus, as infection is shown to be transmissible by two other species of aphis. At the same time, owing to the extreme facility with which *M. persicae* transmits leaf-roll, the writer is of opinion that there exists some kind of affinity between the two. The experiments described herewith and carried out over three years, were designed to give information on the following four points:

(1) The duration of the waiting period, if any, of the leaf-roll virus within the body of the aphis.

(2) The incubation period of the leaf-roll virus in the plant.

(3) The time necessary for a non-infective aphis to feed on a leaf-roll plant to pick up the virus.

(4) The time necessary for an infective aphis to feed upon a healthy plant in order to infect it with leaf-roll.

The experiments were of two types, designed as follows: in the first type a leaf-roll British Queen potato plant, used as the source of infection, was colonised with a number of known healthy apterous viviparous females of *M. persicae*. These aphides were allowed to feed for a definite time, a period which was kept constant throughout the particular experiment. They were then transferred in separate lots to sprouted half-tubers of President and Arran Victory and allowed to feed for certain definite periods. For example, in the first series of experiments, the constant time of feeding on the leaf-roll source was 2 hours, while the times on the healthy half-tubers ranged from 2 hours to 7 days. As will be seen from the details of the experiments, the periods of feeding on the leaf-roll source were 2, 6, 48, 96, 144 hours and 7 days, while the times of feeding on the healthy half-tubers were 2, 6, 12, 24, 48, 72, 96, 120 hours and 7 days. The first experiment, then, consisted of six series of inoculation tests in which the aphis had fed for 2 hours on the leaf-roll source and the above-mentioned periods on the healthy half-tubers. In the second experiment, the constant period of feeding on the leaf-roll source was 6 hours with seven series of inoculation tests; in the third experiment 48 hours and so on. It is clear that if the aphis has fed for

2 hours on the source of infection and all the experiments are negative, then 2 hours' feeding is not a sufficient time to allow the insect to pick up the virus. Again, if infection develops first only in the 6/48-hour series and not before, it is plain that 6 hours are sufficient for the aphis to pick up the virus, but that a period is required longer than 6/24 *i.e.* 30 hours before the insect is infective to a healthy plant. Although these experiments are fairly straightforward, yet they presented considerable difficulty, especially those dealing with the short periods of feeding. There could be no guarantee in these latter cases that the aphis had fed upon either source of infection or experimental plant during the short periods, as *M. persicae* exhibits a strong tendency to wander away temporarily from its food plant if conditions of temperature and humidity are not suitable. It therefore became necessary to repeat the experiments many times before success was achieved.

The results of the first type of experiment were as follows: all tests in which the constant time of feeding on the leaf-roll plant was 2 hours were negative, no matter for how long a period the aphides were subsequently left on the healthy plants. This seems to show that 2 hours' feeding on a leaf-roll source is not a sufficient time to allow the insect to pick up the virus. Positive infections first appeared in the series where the constant time of feeding on the leaf-roll source was 6 hours and where the period on the healthy plant was 48 hours, making a total of 54 hours. All the 6/24-hour experiments, *i.e.* those with a total of 30 hours, were consistently negative. In the tests entailing a total period of more than 54 hours positive infections were fairly frequent and regular. Further, it was found that in those experiments which had a long period on the source of infection, *e.g.* 7 days, or where the aphis was known to be infective, then 2 hours' feeding on the healthy plant was sufficient for the leaf-roll virus to be transmitted to that plant. From these experiments, then, the following facts seem established:

(1) The *non-infective* aphis can pick up the leaf-roll virus after 6 hours' feeding on a leaf-roll source.

(2) The *infective* aphis can transmit the leaf-roll virus to a healthy potato plant after 2 hours' feeding.

(3) The whole process whereby a non-infective aphis picks up the leaf-roll virus and infects a healthy plant cannot be performed in 8 hours, a minimum period of approximately 54 hours is necessary.

(4) The incubation period of the leaf-roll virus in the plant averages 14 days under the writer's conditions.

Further details of these experiments are given in Table I.

Table I.

This table gives the dates and times of the experiments bearing on a possible incubation period of the leaf-roll virus within the body of the aphis vector. Note that all experiments are negative which have a total feeding period, on leaf-roll source and healthy plant, of less than 54 hours.

	Time on source of leaf-roll infection	Time on healthy plants	Dates of duration of experiments	Appearance of first symptoms	Incubation period in plant (days)	Mean daily temp. (° F.)
I	2 hours	2 hours	June 5	—	—	66
	,,	6 ,,	June 5	—	—	66
	,,	12 ,,	June 6	—	—	65
	,,	24 ,,	June 6–7	—	—	65
	,,	48 ,,	June 7–9	—	—	66
	,,	7 days	June 7–14	—	—	66
II	6 hours	24 hours	June 22–23	—	—	66
	,,	48 ,,	July 10–12	July 27	15	74·4
	,,	72 ,,	June 29–July 2	July 18	16	67·7
	,,	96 ,,	July 10–14	July 29	15–17	72
	,,	120 ,,	July 10–15	July 23	8–13	73
III	48 hours	48 hours	March 7–9–11	March 27	16	67
	,,	96 ,,	March 7–9–13	March 27	14	67
	,,	7 days	March 7–9–16	March 27	11–18	67
IV	96 hours	24 hours	March 7–11–12	March 29 and 31	17 and 19	67
	,,	48 ,,	March 7–11–13	March 29	16–18	67
V	144 hours	48 hours	March 13–19–21	April 3	13–15	64·7
	,,	72 ,,	March 13–19–22	April 6	15–18	64·1
VI	7 days	(i) 2 hours	June 19–26	July 8	12	65
	,,	(ii) 2 ,,	June 27–July 4	July 14 and 17	10 and 13	65
	,,	6 ,,	June 1–7	June 17	10	65
	,,	24 ,,	June 3–10	June 21	11	66
	,,	48 ,,	June 3–10–12	June 21	9	66
	,,	96 ,,	June 3–10–14	June 20	6–10	66
	,,	120 ,,	June 3–10–15	June 21	6–11	66

The second type of experiment was based on the fact that the aphis *M. persicae* does not lose its infective power after feeding on a number of plants without again having access to a source of leaf-roll infection. These experiments were performed as follows: after 6 hours' feeding on a leaf-roll plant, the aphides, twelve in number, were transferred to the sprouted half-tuber for 24 hours. They were then removed to a second healthy half-tuber for a like period, then to a third and so on. The half-tubers colonised directly from the leaf roll source giving a total of 30 hours, *i.e.* 6/24, remained healthy, but those giving a total of 54 hours developed leaf-roll in a few cases, while the half-tubers further on in the series, *i.e.* the 3rd, 4th and 5th sets, developed leaf-roll with fair regularity. This second type of experiment gave much the same results as the first, indicating as before that there is a waiting period of approximately 54 hours before the insect can become infective to a healthy

plant. It is of interest in view of the experiments described in Section III of this paper to mention that it occasionally happened that the plants in, say, the 4th and 6th set developed leaf-roll, while those in the 5th set remained healthy, thus showing that it is sometimes possible for leaf-roll infective aphides to feed on a healthy plant without transmitting the virus.

From the details of the experiments given in Table I it will be seen that infection developed in all cases where the total period of feeding exceeded 54 hours. The incubation period of the virus in the plant averaged about 14 days; it does not seem possible to correlate the length of the incubation period of the virus in the plant with temperature in these experiments.

It is of interest, however, to find that this incubation period is shortest in the series where the aphides were 7 days on the source of leaf-roll infection. It is possible that there is some relationship between the short incubation period of the leaf-roll virus in the plant and the long period of feeding of the insect vector on the leaf-roll source. McClintock and Smith (7) in studies upon spinach blight, report a similar occurrence. They state (p. 40): "the longer the aphides remained on the diseased plants before being transferred, the shorter the time until visible symptoms of blight appeared on the inoculated seedlings."

In the second type of experiment, where the infective aphides after 6 hours on the source of infection were passed progressively from healthy plant to healthy plant after 24 hours on each, infection commenced to develop in the second set of plants with a total feeding period of not less than 54 hours. The incubation periods of the leaf-roll virus in the plant in these experiments were 16, 15 and 16 days respectively.

There are at least two possible aspects from which to view this waiting period of 54 hours before the aphis is infective. It may either be considered as a period while the infective principle of leaf-roll undergoes an obligate change within the body of the aphis, in other words it is a true incubation period; or it may be looked upon as merely the time necessary for the virus to pass down the gut, diffuse into the blood of the insect, and finally to return to the saliva via the salivary glands and thence into the plant. This is apparently a necessary procedure, as the oesophageal valve possessed by aphides and many Homopterous insects effectually precludes any regurgitation of infective plant sap. In an attempt to throw some light on this latter point an effort was made to induce the aphides to absorb some staining material while feeding, in the hope that the passage of the particles of dye might be followed

through the alimentary canal and the time thus taken for their passage correlated with the waiting period of the virus in the insect. These experiments were not very successful and their failure may perhaps be attributed partly to the use of the wrong dye. The procedure was as follows: shoots of healthy potato were placed in an aqueous solution of eosin, and when the stain appeared in the leaves, these were colonised with virus-free individuals of the aphis *M. persicae*. The passage of the stain into the body of the aphis was very rapid and in less than 24 hours the whole insect including the feet was stained with the eosin.

In this connection it is of interest to refer to some experiments by Swezy (16), which were performed with a similar object in view, using *E. tenellus* Baker, the vector of curly-top of the sugar-beet. Swezy used the stains, eosin, methylene blue and trypan blue, methylene blue in sugar solution proving the most suitable. At room temperature at the end of 5 hours or even less, the salivary glands of the leaf hopper contained granules stained with methylene blue. Here again, it does not seem possible to correlate the movement of the dye with the waiting period of the virus in the insect vector, which in the case of *E. tenellus* and the virus of sugar-beet curly-top is not as a rule less than about 24 hours.

It is unlikely that the period of 54 hours' delay in the infective power of *M. persicae* is a fixed limit of time, it probably depends greatly on environmental conditions. Elze (4), also working with *M. persicae* and potato leaf-roll, finds the waiting period of the virus in the insect to be between 24 and 48 hours only. The writer, however, obtained consistently negative results with periods of less than 54 hours; possibly this difference may be correlated with varying factors of environment. There seems no doubt, however, that there exists a delay in the development of infective power with *M. persicae* and the leaf-roll virus comparable to similar waiting periods in other insect vectors of plant viruses described by Severin (11), Storey (15), Kunkel (6) and others.

II. Inoculation of healthy plants with the body juices of aphides known to be infected with the leaf-roll virus.

A large number of aphides (*M. persicae*) which had been bred on a leaf-roll source and which by test had been proved to be infected with the virus, were triturated with a few drops of sterile water in a sterile mortar. The liquid was then filtered through muslin to remove the insect fragments and inoculated by needle scratch into a series of healthy potato plants. These experiments proved negative, the plants remaining

healthy. All attempts failed, under the writer's conditions, to infect healthy potato plants with leaf-roll by inoculation with the body juices of leaf-roll-bearing aphides. Previous experiments of a similar nature carried out by the writer (13) with *M. persicae* and other insects and the virus of potato mosaic also gave negative results. There are, however, records in the literature of successful transmission of plant viruses by needle inoculation into healthy plants of the body juices of infective insects. McClintock and Smith (7) produced spinach blight by means of inoculation with the juice of crushed virus-bearing aphides. C. E. Smith (12) reports successful transmission of cow-pea mosaic by needle inoculation with regurgitated sap and abdominal contents from viruliferous beetles (*Ceratoma trifurcata*), and it is also probably possible to transmit curly-top of sugar-beet by inoculation with crushed infective leaf hoppers (Carter (2)). In all these cases, however, the virus is also transmissible by needle inoculation and, apparently, it has yet to be proved whether successful transmission can be accomplished by needle inoculation with juices of infective insects in the case of a virus which is not normally transmissible by needle inoculation such as potato leaf-roll and aster yellows.

III. INFECTION OF A PROGRESSIVE SERIES OF HEALTHY POTATO PLANTS WITH LEAF-ROLL BY MEANS OF VIRUS-BEARING APHIDES.

A series of progressive infections of potato plants was carried out with the object of ascertaining how many healthy plants a given number of infective individuals of this aphis *M. persicae* would infect with leaf-roll without again having access to a source of infection. Three sets of experiments were performed, using in each case 20 individuals of *M. persicae* which had been bred upon leaf-roll plants. The aphides were allowed to feed for periods of 24–48 hours on each set of healthy sprouted half-tubers. In the first experiment infection was carried to the tenth series of plants, in the second experiment to the seventh series and in the third to the sixth series. It is of interest to find, however, that not all the infested plants developed leaf-roll. Occasionally the plants in the third or fourth series, for example, would remain healthy, while those in the second and fifth would become diseased. This occurrence might perhaps be explained on the assumption that the aphides for some reason had not fed on those particular plants, but the writer is of the opinion that it is possible for an aphis bearing the virus of leaf-roll yet to feed upon a healthy potato plant without transmitting infection. Similar occurrences are recorded in the literature. Carsner and Stahl (1) in studies on

curly-top of the sugar-beet state: "a viruliferous leaf hopper may not produce the disease each time it feeds on a healthy plant, even though the periods of feeding be 24 or 48 hours or even longer." Samuel *et al.* (10), working with spotted wilt of tomato and the thrips *Frankliniella insularis*, find that infective individuals fed for successive days on fresh healthy tomato plants did not infect every plant on which they fed.

IV. Transmission of American leaf-roll by *M. persicae* Sulz.

In order to study the relationship of *M. persicae* to the leaf-roll virus as it exists in America, some inoculation tests were carried out with this insect and a leaf-roll Green Mountain potato plant from America, kindly supplied by Dr Schultz. A series of transmission experiments were carried out from this plant to President and Arran Victory potatoes. All the infested plants developed leaf-roll normally and behaved similarly in every way to those infected by *M. persicae* from the writer's own stock of leaf-roll plants. It would appear from this small test that potato leaf-roll as it occurs in America is as easily transmitted by *M. persicae* as the English strains of this virus.

V. On some additional aphis vectors of the leaf-roll virus.

A number of other aphides have been subjected to careful tests in order to discover what species besides *M. persicae* may possess the power of transmitting the virus of leaf-roll. Of five additional aphis species tested, three habitually infest the potato plant while two are confined to glasshouses and in the British Isles occur only occasionally in the open. The three potato-feeding aphides are as follows:

Macrosiphum gei Koch (= *solanifolii* Ashm.);
Myzus pseudosolani Theob.;
Aphis rhamni Boyer (= *A. solanina* Pass. and *A. abbreviata* Patch.).

Macrosiphum gei has been tested for four consecutive seasons and has not yet transmitted leaf-roll in a single instance. *Aphis rhamni* has been tested for two seasons with similar results. *Myzus pseudosolani*, on the other hand, tested for the first time this season (1930), has transmitted leaf-roll in a few cases. Out of 31 inoculation tests with three potato varieties, four plants developed leaf-roll in 18 and 21 days. This aphis, however, does not appear to possess the same affinity for leaf-roll as does *M. persicae*. Murphy and M'Kay (8) report positive infection with *M. pseudosolani* and potato leaf-roll in a small number of cases.

The two glasshouse aphides used in these tests were *Aphis gossypii* Glover and *Myzus circumflexus* Buckt. The tests with *A. gossypii* gave

negative results, but *M. circumflexus* proved capable of transmitting leaf-roll. Out of a total of 21 inoculation tests, 7 plants developed leaf-roll in periods ranging from 12-26 days. Whitehead (17) has also shown, independently, that this aphis can act as a vector of potato leaf-roll. These experiments show, therefore, that two other species of aphis are able to transmit the virus, and the fact that one of them is a potato-feeding species is of some importance. Probably, if systematic tests were made of all the species of aphides which, under experimental conditions, would feed upon potato, other species capable of transmitting the leaf-roll virus would be discovered.

It is clear then that no absolute affinity exists between *M. persicae* and potato leaf-roll, but that this aphis appears to be specially suited in some way to its dissemination cannot be denied. It is very difficult at the present stage to suggest any good reason why one species of aphis should be a more efficient vector than another, and in the hope that some further information might accrue, the writer has repeated part of an earlier investigation on the feeding methods of the aphides (14). The stylet tracks of *Macrosiphum gei* and *Myzus circumflexus* in the tissue of the potato plant were traced out afresh and compared with that of *A. rhamni* which had not been so investigated. The experiments showed, however, that all three species were phloem feeders and, while *M. circumflexus* transmits the leaf-roll virus, *A. rhamni* and *M. gei* have consistently failed to do so. There appears therefore to be little hope of correlating power of leaf-roll transmission with methods of feeding. Again, the fact that *M. circumflexus* and *M. pseudosolani* are capable of disseminating leaf-roll is of interest in its bearing on a possible relationship between the toxicity to the plant of an insect's saliva and its ability to transmit virus diseases. It is a fairly reasonable assumption to suppose that if the saliva of an insect is very toxic to the plant, then that insect is less likely to act as an efficient virus transmitter, owing to the local disorganisation of the plant cells at the point of entrance of the insect's stylets. If the virus, on injection, is surrounded by a mass of dying and disintegrating cells, the likelihood of its successful establishment seems considerably lessened. Some such phenomenon has appeared to the writer to be the reason why capsid bugs seem to play such a small part in virus transmission. Certain of these insects have exceedingly toxic saliva, the place of puncture in the plant being marked by a patch of dead cells (14). Both *M. circumflexus* and *M. pseudosolani*, but particularly the latter, produce greater visible disturbances in the plant tissue when feeding than does *M. persicae*. *M. pseudosolani* induces a marked curling or rosetting of

the shoots of the potato (Plate XI, fig. 3), and when the number of aphides is large, the effect may simulate that of the virus disease "curly dwarf." When *M. pseudosolani* is colonised upon tobacco seedlings the local disturbance caused in the leaf tissue by the insect's saliva is very great. Yellow spots develop at the stylet puncture (Plate XI, fig. 2), the leaves become distorted and the petiole twisted upon itself in the manner shown in Plate XI, fig. 1. Such leaves do not appear to recover, but the damage is not systemic and the new leaves develop without blemish. It appears therefore that toxicity of an insect's saliva as measured by local disturbances caused in the cells of the host plant does not influence very largely the insect's power to transmit plant viruses. There is still, however, this difference in the toxic effect produced on the plant by the saliva of aphides and capsid bugs respectively, that while in the case of aphis punctures malformation and spotting of the leaves occur, the saliva of the capsid bug actually kills outright a patch of cells at the point of entry of the stylets and this fact may still influence virus transmission by this class of insect.

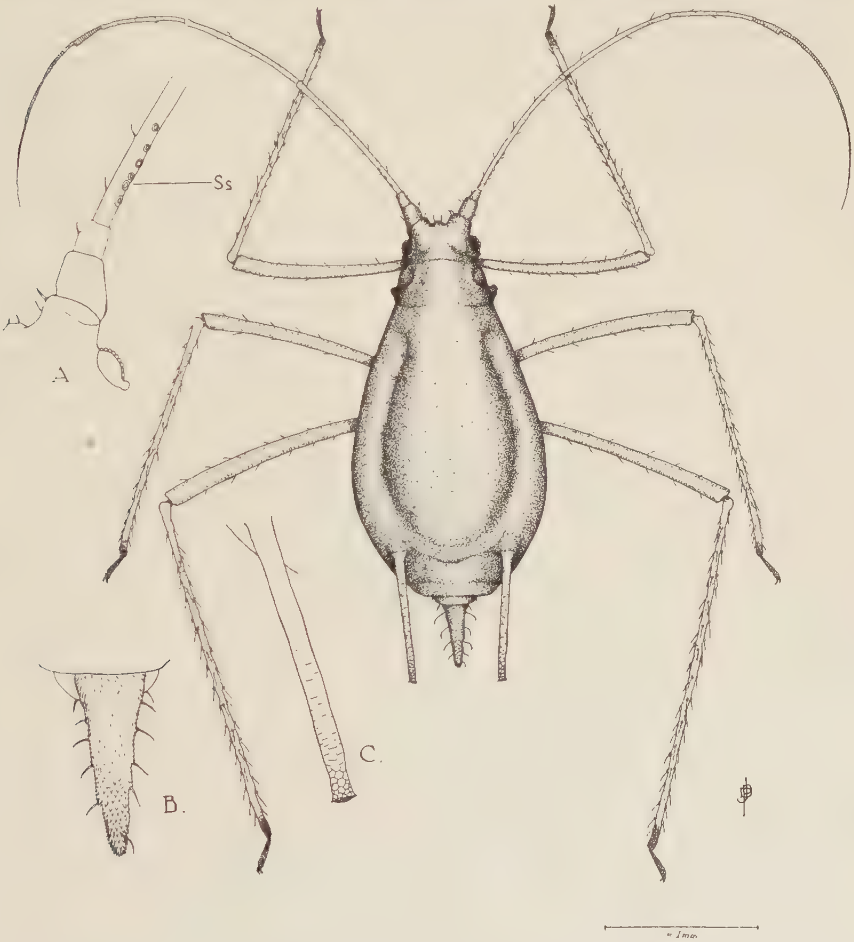
VI. The identity and chief characteristics of the common potato-feeding aphides in the British Isles.

This seems a suitable occasion to record the identity of the common potato-feeding aphides of Great Britain and indicate some of the diagnostic characters. These aphides are four in number, *i.e. M. persicae* Sulz., *M. pseudosolani* Theob., *A. rhamni* Boyer. (= *A. solanina* Pass. and *A. abbreviata* Patch.) and *Macrosiphum gei* Koch (= *solanifolii* Ashm.), and they are illustrated in Text-figs. 1–5. Of these four the writer has found that two, *Myzus persicae* and *M. pseudosolani*, will transmit leaf-roll under his conditions. *M. circumflexus* Buckt., a glasshouse species, will also disseminate leaf-roll as shown in this paper, and an illustration is given of this insect in addition (Text-fig. 4).

Macrosiphum gei *Koch* (solanifolii *Ashm.*).

This is rather a large aphis, the general colour green, often with a slight mealy coating in the nymphal stages. The head is darker with a narrow dark border along the hinder margin, thorax dark green to brownish, abdomen yellowish green, ventral surface light green. In the winged viviparous female, segment 3 of the antennae has about 14 sensoria in a row along its inner face, and in the apterous viviparous female the same antennal segment has a few (3–6) round sensoria, usually on the inner face near the base. The cornicles of *Macrosiphum gei* afford a

good means of identification. These are rather long, tapering distally and reticulate at the distal end for about one-fifth of their length. Length 4·0–4·1 mm. Davidson (3) gives a good description of this species.



Text-fig. 1. *Macrosiphum gei* Koch (*solanifolii* Ashm.). Apterous viviparous female. ×20. A, head and antenna; B, cauda; C, cornicle. ×45. *Ss*, sensoria. Note reticulation of cornicles.

Myzus persicae *Sulz.*

Apterous viviparous female. Rather small, ovate, shiny, of various shades of green, yellow or rose; head broad, frontal tubercles prominent, antennae greenish and slightly shorter than the body; legs greenish,

apices of tibiae and tarsi dark; cornicles slightly swollen, tips dusky. Length 2–2½ mm.

Alate viviparous female. Head and thorax black; abdomen greenish with one or two transverse dark bands, a large dark patch between the cornicles and four lateral dark spots; cornicles dark and slightly swollen towards the middle, cauda dark and pointed; legs pale yellow, greenish or reddish, femora and apices of tibiae dark, tarsi black; third joint of

Text-fig. 2. *Myzus persicae* Sulz. Apterous viviparous female. ×20. A, head and antenna; B, cauda; C, cornicle. ×45. Note slight swelling of the cornicles.

antennae tuberculate with a number of sensoria; wings rather large. Length 2–2·5 mm. An important characteristic of *M. persicae* is the possession of slightly swollen cornicles. For further details of this aphis, see Theobald, *Aphididae of Great Britain*, I, 320.

M. pseudosolani *Theob.*

Very similar to *M. persicae*, but it can be differentiated by the pale cylindrical cornicles and, in the apterous female, by the presence of one or two sensoria at the base of the third antennal segment (See Text-

fig. 3 A). The frontal tubercles are less pronounced than in *M. persicae* and the apices of the cornicles more widely flared and darker at the tips. The insect is slightly larger than *M. persicae* and the legs are longer. See also Theobald, *op. cit.* I, 313, and Patch (9). Length 2·5–2·8 mm.

Text-fig. 3. *Myzus pseudosolani* Theob. Apterous viviparous female. ×20. A, head and antenna; B, cauda; C, cornicle. ×45. Note cylindrical cornicles.

M. circumflexus *Buckt.* (= M. vincae *Gill.*).

Apterous viviparous female. Bright, shiny, yellowish-green in colour; thorax with one or two dark spots on each side; abdomen with dark patches, one of irregular horseshoe shape and one large black patch, these markings may be entirely absent; antennae yellow to green, apices of segments 3–5, and all of segment 6, dusky, frontal tubercles straightly prominent, antennal segment 3 longer than 4 with a sensorium near the

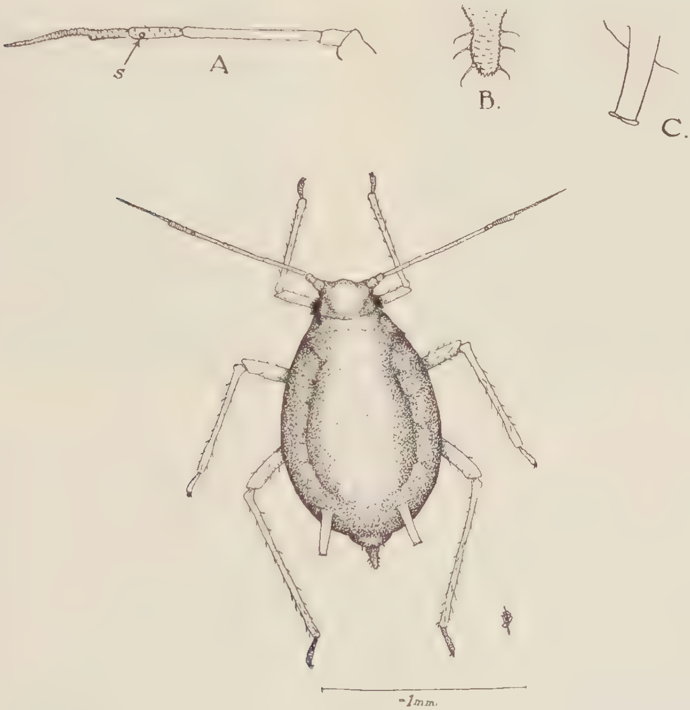
base, cornicles yellow, rather long and thin, in some dusky at apices. Length 1·6–1·8 mm. (Theobald, *op. cit.* I, 332–333.)

Text-fig. 4. *Myzus circumflexus* Buckt. Apterous viviparous female. ×27. A, head and antenna; B, cauda; C, cornicle. ×60.

Aphis rhamni *Boyer* (A. solanina *Pass. and* A. abbreviata *Patch*).

Apterous viviparous female. Pale green to pale yellow, colour variable; antennae green or yellow with apices dusky; cornicles cylindrical, short, straight and rather stout, apices darker, cauda rather short and thick with three hairs on each side. In the apterous viviparous female there is a single sensorium on the fifth antennal segment; proboscis reaches to, or just beyond, the second coxae; legs with short spine-like hairs on tibiae, tarsi dusky. Length 1–1·2 mm. (Theobald, *op. cit.* II, 199–202.)

The drawings of the five species of aphides in Text-figs. 1–5 were prepared by Mr J. P. Doncaster, to whom the writer is greatly indebted. Acknowledgment is also due to Mr F. Laing for his kind assistance with



Text-fig. 5. *Aphis rhamni* Boyer (= *A. solanina* Pass. and *A. abbreviata* Patch). Apterous viviparous female. ×27. A, antenna; B, cauda; C, cornicle. ×60. *S*, sensorium. Note that the sensorium is usually found on the 5th antennal segment. Its presence on the 4th, as shown here, is exceptional and is due to the lack of an antennal segment in this particular specimen.

the identification of various aphides, to Miss M. E. Sewell for her care of many of the plants used in the experiments, and to Dr H. W. Miles, who supplied the writer with the aphis *M. circumflexus* used in the 1929 experiments.

VII. Summary.

1. Experiments bearing on a possible relationship between the leaf-roll virus and the aphis vector, *M. persicae* Sulz., are described.

The following facts are elucidated by the experiments:

(*a*) The *non-infective* aphis can pick up the virus of leaf-roll from an infected potato plant after 6 hours' feeding.

(*b*) The *infective* aphis is capable of transmitting the leaf-roll virus to a healthy potato plant after 2 hours' feeding.

(*c*) The whole process whereby a non-infective aphis picks up the leaf-roll virus and infects a healthy plant cannot be performed in 8 hours; a minimum period of approximately 54 hours appears to be necessary.

(*d*) The incubation period of the leaf-roll virus in the potato plant, *i.e.* from the time of infection to the time of the appearance of first symptoms, averages 14 days under the writer's conditions.

2. It has not been found possible to infect healthy potato plants with leaf-roll by needle inoculation with the body juices of infective aphides.

3. Infection of progressive series of healthy potato plants with virus-bearing *M. persicae* showed that the aphides were capable of infecting respectively 6, 7 and 10 healthy potato plants without again having access to a source of leaf-roll infection. It is noteworthy that occasionally plants in the progressive series of infections failed to develop leaf-roll, although plants before and after in the series became infected.

4. The leaf-roll virus occurring in American potato varieties as exemplified by "Green Mountain," is as easily disseminated by the aphis *M. persicae* as the leaf-roll virus occurring in the British Isles.

5. Further inoculation tests with five additional species of aphides are described; of these five aphides, two were proved to be capable of transmitting the virus of potato leaf-roll. These are the potato aphis, *M. pseudosolani* Theob. and the greenhouse aphis, *M. circumflexus* Buckt. Negative results were obtained with two other potato aphides, *i.e.* *Macrosiphum gei* Koch and *A. rhamni* Boyer, and one greenhouse aphis *A. gossypii* Glover.

6. Illustrations are given of the four chief potato-feeding aphides in England, together with the greenhouse aphis *Myzus circumflexus*, which has been proved capable of transmitting leaf-roll. The chief diagnostic characters of these five aphides are indicated.

Fig. 1.

Fig. 2.

Fig. 3.

SMITH.—Studies on Potato Virus Diseases (pp. 141–157).

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EXPLANATION OF PLATE XI

Fig. 1. Leaf of White Burley tobacco plant, showing the twisting of the petiole caused by the feeding of the aphis *M. pseudosolani*.

Fig. 2. Leaf of White Burley tobacco plant, showing the bright yellow spots where the aphis *M. pseudosolani* has fed.

Fig. 3. Healthy potato plant showing the rosetting of the growing points caused by the feeding of *M. pseudosolani*.

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GIBBERELLA SAUBINETII (MONT.) SACC. ON BRITISH CEREALS

II. PHYSIOLOGICAL AND PATHOLOGICAL STUDIES

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(With Plates XII–XIV and 2 Text-figures.)

CONTENTS.

PHYSIOLOGICAL STUDIES OF *G. SAUBINETII*.

THIS paper is a continuation of one previously published (4) that dealt with the isolation, and the cultural and diagnostic characters of *G. Saubinetii* (Mont.) Sacc. as occurring on British cereals. It was there shown that this organism is allied to the form-genus Fusarium, its conidial stage being *F. graminearum* Schwabe; the various other names applied to it have been fully discussed by Wollenweber (11). Whilst *G. Saubinetii* has been studied intensively in Europe and America, it has not previously been investigated in this country. The physiological studies recorded in this paper are limited to those bearing upon the occurrence of the Gibberella disease in normal British farming. The pathological studies (p. 170) are directed more particularly towards a comparison with certain

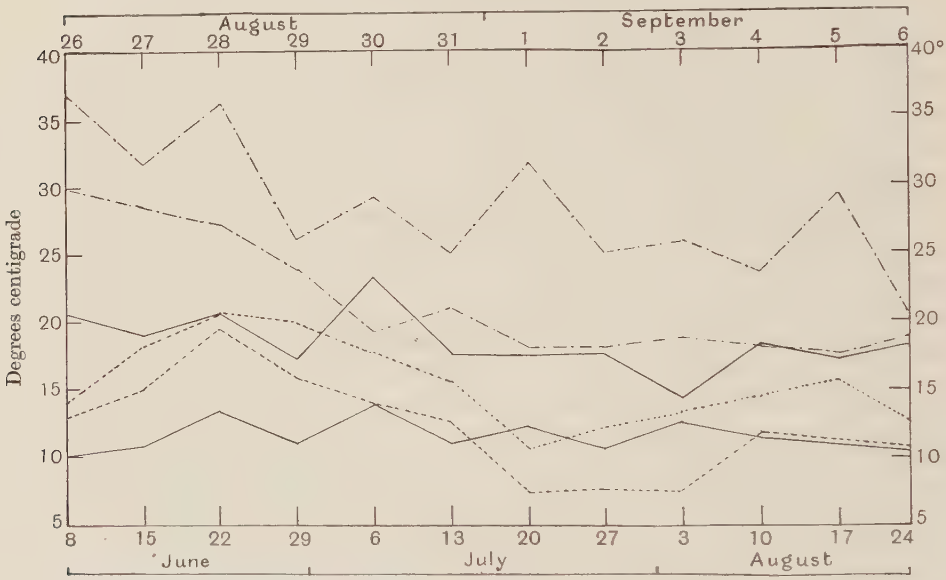
Fusarium species from the point of view of virulence and symptoms, since *G. Saubinetii* in its Fusarium stage occurs on cereals most frequently in conjunction with *F. culmorum* and *F. avenaceum*, disease-causing organisms that have previously been described (5).

Production of perithecia; requisite conditions.

G. Saubinetii produces mature perithecia readily when cooked wheat grains, inoculated from pure cultures of the conidial phase and kept in tubes until growth has developed, are put into moist Petri dishes or on moist, sterile soil, and kept at a suitable temperature. Living grains of wheat, barley, oats and rye, infected naturally outdoors or by artificial inoculation from pure cultures, soaked in water and placed under similar conditions, produce a mycelium and conidia first and perithecia later. The perithecia may be borne singly or in very small clusters either on the sparse aerial mycelium or directly on the husk; but on wheat and rye they frequently occur in crust-like masses on the grain below a rather dense mat of mycelium that masks them. The length of time required for the production of perithecia on infected grain varies from about two weeks to two months; the shortest period observed was when barley grains from ears inoculated whilst growing, soaked in water and put into Petri dishes on May 25, bore mature perithecia by June 6, the temperature range having been 12° to 28° C., mainly 17° to 25° C. Ears of wheat and barley inoculated whilst growing (August), and placed when cut (September 18) in moist jars standing inside a south window, produced perithecia containing ascospores by September 30; the temperature inside the chambers ranged from 15° to 30° C., the average minimum and maximum being 16° and 27° C. respectively. During this same period ears left on the plants outdoors, enclosed within glass moist chambers, bore occasional, rudimentary perithecia only. The day temperature would be the same in the tubes outdoors as in the jars indoors, yet the "heat-hours" outdoors would be considerably less. The jar test, repeated in October when the temperature range was 8° to 30° C., with average minimum and maximum 11° and 24° C. respectively, yielded incipient perithecia only; a parallel test carried out at a constant temperature of 22° C. yielded mature perithecia.

These results led to the following method of ascertaining within narrower limits the temperature range determining the production of ascospores within the perithecia. Ears of wheat and barley, inoculated during the summer of 1929, and kept indoors after harvesting, were laid on soil and covered with sacking, from June 2 to August 25: the situation

was sheltered but not shaded, and the ears and soil below were kept moist continuously by overhead watering with air-warmed water. The average minimum and maximum temperatures in the shade were: June, 11·1° and 19·5° C.; July, 12·2° and 18·4° C.; August, 12·2° and 19·5° C. The temperature reached 27° to 29° C. on two successive days, 25° C. on two other successive days, and 25° C. on one day; at no other time did it reach 25° C. By June 30 perithecia were abundant, but none



Text-fig. 1. Temperature range for the production and maturation of perithecia.

———— Summer shade temperature; average weekly maximum and minimum during June, July, August.

- - - - - - - Maximum and minimum daily temperature, August 26–September 6; perithecia did not mature at this range.

—.—.— Maximum and minimum daily temperature, August 26–September 6; perithecia did mature at this range.

contained ascospores, nor had they developed by August 25. On this date the ears bearing these immature perithecia were divided into two sets; one remained outdoors as before, whilst the other was placed in the laboratory in a south window and the containing box covered with glass; other conditions were alike for both. By September 7 the ears indoors bore numerous mature perithecia, some containing germinating ascospores, others having discharged these spores. Outdoors the perithecia contained no ascospores, the contents consisting of unorganised matter rich in oil. Text-fig. 1 shows graphically these relative tem-

perature ranges, (*a*) in shade outdoors during summer, (*b*) at which perithecia do not mature, and (*c*) at which they do mature. The curves show that the outdoor summer range corresponds approximately to that under which perithecia did not mature, other conditions being favourable. This summer range is considerably lower than that at which perithecia matured, and only for very brief periods reaches this latter range at all. Thus normal summer shade temperature suffices for the production of immature perithecia, but not for the production of ascospores. For this purpose a day temperature range of about 25° to 30° C. is necessary; or, if the nights are cold, the day temperature may counterbalance this by reaching 35° to 40° C., providing such high temperature be not prolonged. It is essential that the mean daily temperature be not less than 21° to 22° C.

The second essential factor is a sufficiency of moisture. In the tests here recorded the atmosphere was saturated, but Petri dish tests show that this degree of humidity is not necessary constantly; occasional saturation only is required, provided that the perithecia are not subjected to desiccation during the period of spore development and ripening.

Such periods of high temperature and humidity probably occur in parts of European and Asiatic Russia where the mature perithecial stage occurs in nature, and also in parts of the U.S.A. where, according to Dickson (6), "51 per cent. of the infected specimens examined showed perithecia in the wet season of 1919." That such perithecia have not been observed in the British Isles is good evidence that they are rarely, if ever, produced here. There is no question as to the ability of the British strain of the fungus to produce perithecia under suitable conditions; their absence under natural conditions is clearly due to the fact that British climatic conditions provide neither a sufficiently high shade temperature nor sufficient moisture when the shade temperature is occasionally high enough.

Reference must be made to one other point concerning the production of mature perithecia. With the exception of cooked wheat, the substrata referred to above were natural materials generally much contaminated with various micro-organisms. The presence of such organisms is not, however, a necessity, though Atanasoff (2) states that some unidentified bacteria stimulated the production of perithecia. With sufficient moisture and a suitable temperature, *G. Saubinetii* produces mature perithecia freely on sterile artificial media, such as oat-, wheatmeal-, potato- and glycerine-agar, from both single conidium and single ascospore sources.

Minimum temperature for germination of spores.

The proportion of conidia and ascospores which germinate in water, and the rapidity of germination, depend upon the age and maturity of the spores as well as upon the temperature. In a single perithecium there are ascospores in different stages of maturity, but in a completely developed perithecium most of the ascospores are mature and would be discharged naturally. On placing such a perithecium in a drop of water the ascospores are discharged immediately, without applying pressure, and about 90 per cent. of them germinate within twelve hours at 10° to 12° C. From 75 to 90 per cent. of freshly formed conidia germinate within twenty-four hours at normal summer temperature, *i.e.* between 12° and 20° C. For both ascospores and conidia the rate of germination is gradually, but only slightly, reduced as the temperature falls from 12° to 9° C., and more markedly so from 9° to 5° C.

Table I. *Germination of spores at certain temperatures; expressed as a percentage number.*

	Ascospores			Conidia		
Temp. ° C. ...	4 to 8		8 to 12	4 to 8·9		8·9 to 12
End of ...	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
Trial *a*	1	10	75	0	75	80
,, *b*	1	10	40	30	75	82
,, *c*	5	50	75	25	60	75

The figures show that there is definite, though slower, germination below 9° C., and that the reduced rate is due to lower temperature, not to lack of vitality. The approximate minimum temperature for germination as ascertained by trials within a range of 3·3° to 6° C. was found to be about 5° C. At a temperature continuously below 10° C. the spores not only germinate, but they develop a mycelium both in water and in nutrient solutions. The bearing of this on infection of host plants will be mentioned later (p. 164).

Resistance to low temperature; overwintering; longevity.

G. Saubinetii is extremely resistant to low temperatures in both its vegetative and perithecial phases. Pure cultures on cooked wheat, in the vegetative stage withstood a temperature that was reduced every third day to − 20° C. for forty-five days, and that during this period never reached 0° C. After subjection to this low temperature the fungus, under suitable conditions, grew vigorously and produced perithecia more readily and abundantly than other parts of the same cultures kept at

normal temperatures. Ascospores from perithecia submitted to similar low temperature for thirty-five days germinated as speedily and readily (90 per cent. in two days at about 12° C.) as did untreated ascospores. It is evident that the fungus is not adversely affected by temperatures much lower than those occurring in winter in our climate.

Overwintering under natural conditions, however, involves variation of temperature and moisture, hence the capacity for overwintering was investigated in another way. Cooked grains bearing pure culture growth, and ears of wheat, barley and oats, that had been artificially infected during growth in summer, were left outdoors in wire baskets from November to March. This exposed material yielded the fungus again in most cases when incubated. On poor wheat and oat ears, and on straw, the fungus generally produced a few conidia only, indicating a very low degree of vigour; occasionally the Gibberella was almost, or completely, crowded out by saprophytic fungi and bacteria. Diseased barley grains, on the contrary, were less affected by foreign organisms, and the Gibberella grew vigorously in spring, producing either an obvious mycelial growth or abundant conidia. Thus Gibberella in its vegetative state retains vitality outdoors through winter, but its vigour is greatly reduced in competition with saprophytes, and under ordinary field conditions much material is undoubtedly thus rendered innocuous.

Since ascospores are not produced under natural conditions in this country their capacity for overwintering is of less moment, but their behaviour may usefully be mentioned here. Ears of wheat and barley bearing mature perithecia were exposed to outdoor winter conditions as described. They were examined when milder weather began early in April, but it was then found that the ascospores had, for the most part, been discharged. Those still present in the perithecia were shrunken, or showed signs of germ-tubes which had ceased growth immediately after protrusion. Of these residual ascospores not one in a thousand showed signs of vitality, whilst those that made some attempt to germinate yielded nothing more than a slight outgrowth which never developed into a hyphal stage in water or nutrient solutions. Ascospores from similar material kept indoors during winter, and tested in parallel with those wintered outdoors, germinated freely and rapidly. Evidently perithecia produced and matured in summer discharge their ascospores before spring, and spring infections in our crops would arise mainly, or entirely, from sources other than ascospores even if such spores were produced naturally. In the U.S.A., according to Adams (1), "one commonly finds on the old corn (*i.e.* maize) stubble in spring the fruiting

bodies of *G. Saubinetii* which no doubt are in part the source of inoculum (*i.e.* of wheat following maize)."

Infection of spring-sown crops may arise from conidia produced in spring on diseased autumn crops and débris, but is doubtless due to a great extent to diseased grain stored in stack or granary until sowing time. According to Maneval (10) some species of Fusarium retained their vitality on stem materials stored in a laboratory for eight years. The Gibberella of the present investigation has been kept two years in naturally infected wheat grains stored in an unheated room, and artificially infected ears of wheat, barley and oats have been stored similarly for one year; after these periods the fungus grew as vigorously as ever. Even the conidia remain viable under reasonably favourable conditions much longer than is generally supposed. Conidia from wheat ears that had remained dry from October to June showed then a germination of 10 to 50 per cent. at 13° to 16° C. within forty-eight hours, but conidia from the same ears remaining indoors for the further period of three summer months were not viable. Conidia on artificial media were found to be viable when the cultures were six months old and had apparently dried out. Thus although conidia under field conditions in summer must germinate within a day or two or perish through desiccation, those under storage conditions may retain their vitality for as long as (or longer than) seven months. Infections in spring-sown crops may, therefore, be due not only to grain containing the fungus internally, but also to conidia disseminated from straw, chaff, grain, etc. Ascospores retain their vitality under storage conditions longer than do conidia. Ascospores produced in September on barley ears which were afterwards stored and became brittle with dryness, germinated freely in water at 15° to 19° C. within twenty-four hours a year later. Other ascospores produced in July, the perithecia afterwards remaining on dry filter paper in plugged tubes for fifteen months, were not entirely dead at the end of this time. Under ordinary storage conditions ascospores would, no doubt, retain their vitality for at least one year, and probably much longer; and though the perithecial stage does not assist the organism to persist through winter, it could do so through periods of drought.

Temperature for infection of host plants.

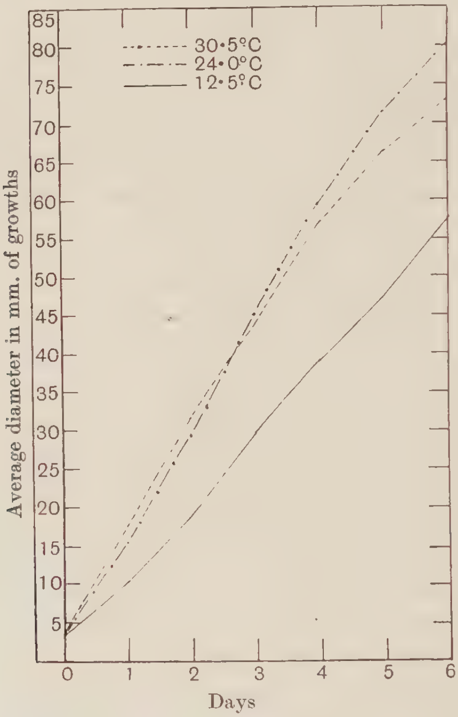
It is well known that our cereals, more especially wheat, make some amount of growth both below and above soil level at temperatures between 5° and 10° C. In the foregoing pages it has been shown that both conidia and ascospores germinate and produce mycelia below 10° C.,

whilst ascospores were discharged during mild periods in the winter. The temperature range during this winter was: Nov. – 3·3° to 12·2°; Dec. – 2·2° to 12·8°; Jan. – 2·8° to 12·2°; Feb. – 3·3° to 7·2°; March – 3·4° to 10°; the average minimum was 1·5° and the average maximum 7·1° C. It has been shown also that this average maximum temperature is higher than the minimum required for the germination of conidia and ascospores. It might, therefore, be expected that at a temperature permitting the growth of plants and germination of spores and growth of the fungus the latter could infect the plants. This was found to be a fact. Wheat seedlings became infected from artificially contaminated grain planted in sterilised soil, and from contaminated soil in which healthy grains were planted, during February and March when the temperature of the house did not exceed 10° C. at any time. Dickson (7) records that *G. Saubinetii* in the U.S.A. does not attack and infect seedling wheat from naturally infected or artificially inoculated seed if the soil temperature is below 12° C. These varying results are not necessarily contradictory, the difference being but one of several which are bound up with differences in "strain" of the organism in different parts of the world. It shows the necessity for the investigation of each "strain" under its own particular conditions.

Rate of growth at various temperatures.

The rate of growth of single conidium and single ascospore individuals on Dox's medium (pH 7) was ascertained at various temperatures. The rate for individuals from conidia and ascospores does not differ appreciably, hence the growths from both of these sources were taken together in arriving at average figures. In order to introduce variation of temperature somewhat comparable to day and night the cultures were kept every night for 12 hours at 11·5° to 13·5° C., and during the day at various temperatures between 12·5° and 45° in darkness. The growth curves in Text-fig. 2 indicate the average diameter on successive days of the individuals growing in Petri dishes. The *maximum* temperature for growth is about 37° C.; each 12-hour exposure to this temperature checked growth and reduced vitality to such an extent that there was no recovery or growth during the ensuing 12·5° period. The rate at 12·5° continuous temperature is shown for comparison. Subjection to 37° C. for seven successive 12-hour periods does not kill the fungus, for it grows afterwards, though slowly, at room temperature; after similar heating at 45° there was no subsequent growth. The *optimum* temperature for growth is about 24° C., a slight increase or decrease in this not more

than counterbalancing the difference in vigour of individuals from different spores. At this temperature the rate of growth for the first few days is slightly greater than at 21° to 22° C., but eventually the rate and amount of growth are alike. At 30° to 31° C., on the contrary, there is still more rapid growth during the first two days, but this is accompanied by loss of vigour with progressively more rapid reduction in the rate; after five days the amount of growth is less than that at



Text-fig. 2. Rate of growth of single-spore individuals at three selected temperatures for successive 12-hour periods alternating with a standard temperature.

lower temperatures. That 24° C. is advantageous in other respects is shown by the appearance of the perithecial stage on the fourth day, and its steady development subsequently. At 30° to 31° C. the first indication of this stage appeared after six days, and subsequent development was distinctly less rapid and less uniform. It is of interest to note that by entirely different methods a constant temperature of 22° C., or a day temperature of about 25° C., was found to be best for the development of the perfect perithecial stage (p. 161). It appears, therefore, that

G. Saubinetii produces perithecia under the conditions most favourable to general growth, so that the perithecial stage is an additional means of reproduction rather than, as generally accepted for many fungi, a means of withstanding adverse conditions. Further, if British conditions do not suffice for the development of perithecia (p. 161), neither do they suffice for the most vigorous general growth of the organism. This fact, together with the absence of ascospores, affords a partial explanation for the lesser prevalence of the fungus here than in some other climates.

By methods similar to the foregoing, and by parallel trials, it has been ascertained that the optimum and maximum temperatures for the growth of *Fusarium culmorum* are 25° to 26° C. and 37° to 38° C. respectively—almost exactly the same as for *G. Saubinetii*. This is not, however, the sole or even the main factor in determining the relative prevalence of these fungi, but it indicates that both will be similarly favoured by similar seasonal temperatures.

Effect of dry heat on the organism within the grain.

The "dry heat" method of treating cereal seed for the control of Gibberella, Fusarium, Helminthosporium, and other seed-borne diseases was investigated by Atanasoff and Johnson (3). After reviewing the work of earlier investigators, they recommended heating the seed for 30 hours at 100° C., the parasitic organisms being thereby killed out, without doing harm to the wheat and barley itself providing these were good samples. In their experiments the grain, without preliminary preparation, was heated in an air oven. The writer tried this method on wheat (Swedish Iron) as received, after threshing, for germination test prior to sowing. At the end of seven days the germination of the unheated seed was 78 per cent., whilst not a single grain that had been heated for fifteen, ten, or even only five minutes at 100° C. in an air oven had germinated in that period. Another trial with sound, well-dried barley, that had been stored in the laboratory for six months after the bulk had been sown in the field, was also disappointing. At the end of seven days the unheated grain showed germinations of 83 (Plumage Archer) and 91 (Svälof Victory) per cent., whilst in samples heated as mentioned for wheat not one grain had germinated. At the end of fourteen days the heated wheat and barley showed either exceedingly low germination or none at all, and fungal and bacterial organisms had developed freely on most grains. Quite apart from the reduction in germination capacity the initial delay is so serious as to rule out this dry heat method of treatment completely. It is admitted that by preliminary drying of

grain (*e.g.* at 45° C. for eighteen hours) and subsequent slow cooling the germination capacity is not similarly affected, but this is not the point at issue, and can be more conveniently dealt with in another report.

The effect of dry heat on Gibberella itself when present on or in grains was investigated in a different way. Diseased wheat and barley grains from ears that had been stored in a dry room for a year, were heated in sterile, dry test-tubes immersed in a boiling-water bath; from each ear equal numbers of grains were heated and left unheated, the latter being the controls. The grains were placed in sterile, lined Petri dishes after heating and left in for from one hour to two days before being moistened for incubation. After incubation every grain was examined microscopically for the presence of Gibberella. The conclusion reached after many trials was that whilst heating for three minutes did not always suppress the fungus, heating for five minutes always killed it. This fact does not, at the moment, appear to be of practical value, in view of the foregoing remarks on the result of heating healthy grains. In the present state of knowledge the "remedy" would be worse than the disease.

Relation of parasite to host plant.

In all affected wheat and barley plants, whether grown in contaminated soil or from contaminated seed, the crown (the part that bears the coronal roots and buds) is the part most thoroughly permeated by *G. Saubinetii*. In contaminated soil the attack on this part is direct and also indirect, viz. from affected roots. The latter become attacked at scattered points, recognised as brown lesions, some being severely affected and killed. Many root tips are also attacked and thus the root system is generally more or less reduced. From artificially contaminated or naturally infected seed the fungus reaches the crown by growth in the mesocotyl or first internode, even when the latter is three inches long. In such plants the coronal roots become attacked at their proximal ends, rarely elsewhere. The congested fungal growth in the crown, together with the damage to the root system, appears to cause the death of seedlings, since the invasion of the stem or culm (mentioned below) is never so general or so extensive as to produce this effect. The fungus extends up the stem from the crown, and is nearly always found in the internode immediately above soil level. The very delicate and sparse hyphae occur in some of the intercellular spaces of the cortex as well as in the parenchyma and the lysigenous cavity of some of the vascular bundles. Associated with the hyphae there is generally a brownish deposit, and this frequently blocks the cavity and one or more of the

vessels of an affected bundle. In stained preparations the deposit is densely coloured, and hyphae have not actually been seen in it. Even in seedlings severely affected at the bases there is no abundant mycelial growth penetrating and permeating the stem tissues as in plants attacked by *Fusarium culmorum.*

In plants growing under unfavourable conditions, *e.g.* close together in small pots, the fungus ascends in the inner tissues (very rarely in the outer sheaths) into the second and even the third internode. The notable feature is that plants so affected look as healthy and vigorous as unaffected, control plants grown under the same conditions. In wheat, grown in small pots, both under glass and outdoors, the fungus ascended the stem beyond the first aerial node in from 50 to 75 per cent. of the plants; beyond the second node in 30 to 40 per cent.; and beyond the third node in 15 to 20 per cent. These figures refer only to plants apparently as healthy and well grown as the controls were, or nearly so. The only plants so grown which reached the stage of bearing small ears never yielded the fungus from the ears or from the rudimentary grains, and no evidence could be found of the passage of the fungus from the stem into the ear and thus giving rise to internal infection of the grains. The observations made support Doyer's (9) statement that "although the internodes of the host plants showed no signs of attack the fungus existed in the parenchyma cells high up in the stalk through the internodes," so far as concerns plants grown under abnormal conditions. Under normal conditions of growth the fungus was never found high up in the stem whilst the plant was in a growing condition, and Atanasoff's (2) view is undoubtedly correct, viz. that there is no growth of the fungus from the base of the plant into the grain as occurs with the Smut fungi. This author's contention that *G. Saubinetii* represents the lowest degree of parasitism, however, appears to be questionable. As compared with *F. culmorum* or *F. avenaceum*, *G. Saubinetii* appears to be the more highly specialised parasite in the sense that it does not so speedily destroy its host, though it attacks cereals as readily as they do, and thrives under equally diverse conditions. This point is made clear in the section dealing with "seedling blight" (p. 170). Again, if barley seedlings attacked by *G. Saubinetii* at their bases are covered with bell jars the fungus frequently emerges to the exterior at parts some distance above the lowest (primary) leaf-sheath, but when the cover is removed the seedlings continue to grow for many weeks nearly as well as uninfected plants. Facts such as these rather indicate, in the opinion of the writer, a considerable degree of specialised parasitism in *G. Saubinetii*.

The general mode of attack on the grains in the ears is described on p. 172. The fungus shows special affinity for the tissues of the embryo, and in the majority of grains that show evidence of early attack the embryo is entirely absent, its place often being occupied by a small, compact mass of mycelium. When infection occurs at a later stage the embryo may or may not be invaded, and if it is, it is not always killed. Such grains when planted give rise to seedlings having discoloured bases, and they generally collapse when only a few inches high. This type of infection is more common in barley than in wheat, owing possibly to the better protection of the embryo by the investing flowering glume of more resistant tissue. In the aleurone layer of the endosperm the hyphae tend to become massed, whilst in the starchy portion they are long, slender and straggling. In shrivelled grains the cells of the endosperm are disorganised, or non-existent; the starch grains, if any, form a loose mass, but are not attacked. In the pericarp the hyphae are restricted, the discoloration of diseased grains being due mainly to the modification of the aleurone cells and contents. Thus diseased grains fall approximately into two main groups; in the first group invasion is from the germ end, the germ is killed, and the mycelium extends to the other parts subsequently; in the second group invasion is not at the embryo, and this may remain unaffected or be but slightly affected, in either case giving rise to a diseased plant if sown.

Pathogenicity of *G. Saubinetii*.

G. Saubinetii has been proved to be pathogenic to wheat, barley and oats; rye was not included in the trials. The experimental methods previously described by the writer (5) were used without modification in order to compare its pathogenicity with that of certain species of Fusarium. Pure cultures of the fungus on cooked wheat grain were used to contaminate sterile soil, a similar amount of sterile cooked grain being added to the control pots. Seed grain was contaminated by contact with pure cultures on artificial media, and inoculation of ears was done by applying a suspension of conidia and mycelial fragments from pure cultures when the ears were in different stages of development.

Effect on seedlings; "seedling blight."

G. Saubinetii attacks cereal seedlings very readily. Seedling wheat, grown (October to December) in an unheated greenhouse and outdoors, under both wet and dry soil conditions, showed all the plants to be infected at the crown, whether derived from healthy seed in contaminated

soil or from contaminated seed in sterilised soil. This extreme proportion of infection was not, however, followed by a corresponding mortality in the seedlings. Under all the above-mentioned conditions there was a full stand of seedlings little or no worse in appearance than the healthy control plants. When seedlings had been grown under adverse conditions, such as with insufficient soil moisture in the greenhouse, in water-logged soil, or under severe climatic changes, as many as 30 per cent. were killed off before the four-leaf stage. The following record is typical, and the plants are illustrated in Pl. XII, figs. 1 and 2, which show a good stand but inferior plants, and in the barley, discoloured bases of the badly affected ones.

Table II. *The relative proportion o infection and mortality in seedlings grown from seed from affected ears.*

Grains from ears artificially inoculated during the growing season; planted on sterilised soil for germination count; then covered with sifted potting soil and grown in a cool greenhouse with temperature range 8·5° to 22° C., average minimum 10, average maximum 17·5° C. No. of grains per pot 25.

	No. germinated	Seedlings above soil	Seedlings in four-leaf stage	Seedlings with basal infection	Seedlings yielding *G. Saubinetii* from aerial part
Wheat					
Not inoculated	24	24	24	0	0
Inoculated	20	19	19	4 (slight)	14
Barley					
Not inoculated	25	25	25	0	0
Inoculated	25	25	23	11	15

The general conclusion from a number of such trials is that, in spite of the frequency of infection, *G. Saubinetii* does not cause "seedling blight" to anything like the same extent as do *F. culmorum* and *F. avenaceum*. Except under extreme conditions of wet, dry, or poor soil, impeding normal seedling growth, *G. Saubinetii*, whether originally in the soil or on the sown seed, will cause comparatively little mortality in seedlings under field conditions. The definite results of infection become apparent, as a rule, at a later stage only. These observations have some bearing on the question of the value of "prolonged germination tests" as a means of indicating the amount of disease of Fusarium type in a sample of seed corn. So far as Gibberella is concerned, seed that will germinate at all will, in most cases, continue growth, in spite of external or slight internal infection (p. 169). Even prolonged germination tests will, therefore, give little additional information as to the presence or absence of such parasite, unless microscopical examination of the grains and incubated shoots be included.

As stated on p. 168, the crown of the plant becomes invaded either directly or indirectly. Any portion of the stem below soil level shows more or less of a brown discoloration; but whilst in barley this frequently extends and is visible above soil level, it is rarely to be seen in wheat and oats. Under similar conditions barley suffers more than do wheat and oats; in addition to the more obvious basal discoloration, there is greater mortality, and the seedlings that continue growth are obviously smaller and more backward than unaffected barley seedlings. In general, only severely affected seedlings that die off early would arrest attention in a field crop; the majority of seedlings affected by *G. Saubinetii* show no distinctive symptoms of attack.

Effect on plants growing to maturity; "foot rot" and its effects.

The diseased crown together with the affected stem base and proximal parts of the roots in plants past the seedling stage is generally termed "foot rot." Throughout the growing period plants so affected are generally smaller and less vigorous than unaffected plants, though not obviously unhealthy. When they reach the mature stage their condition depends upon the intensity of the basal invasion, and this in turn depends upon seasonal soil moisture content and temperature. The plants may appear normal but yield only small sized grain; or, they may "ripen" prematurely and bear few or no grains ("deaf" ears); or, again, the ears may fail to become extruded from the sheaths. The most common results of the foot rot phase are: somewhat earlier ripening, smaller plants and fewer and smaller grains. Barley suffers in these respects more than wheat, and both more than oats. So far as has been observed basal attack does not cause breakage at the foot and falling of the plant, as do basal attacks by *F. culmorum* and *F. avenaceum*. Further, neither failure to extrude ears nor the presence of deaf ears is a common feature, as may be seen from Pl. XIII, figs. 3 and 4. Thus, whilst *G. Saubinetii* is less virulent as a "foot rot" organism than the Fusarium species named it causes reduction in size of grains and yield of crop as they do. Owing to the insidious nature and the obscure symptoms of the seedling blight and foot rot phases of Gibberella disease, it is impossible at present to estimate the damage done to field crops; moreover, the matter is complicated by the frequency with which Gibberella and Fusarium species occur together in nature.

Infection of ears and grain; "ear blight."

The ears of wheat, barley and oats are susceptible to attack by wind-borne conidia of *G. Saubinetii* at all stages from flowering to maturity.

Artificial inoculation, by applying an aqueous suspension of conidia (and inseparable mycelial fragments that pass through folded butter muslin) from a pointed glass tube, was first done during the flowering stage. Every ear so treated and then left exposed in a natural way became infected; in some spikelets a single floret only was affected, in others many or all of them were affected. The difference was due merely to the different position of the inoculum in relation to the floret or spikelet. Infection of a floret by lodgment of inoculum between the flowering glume and pale, on the edges of these, or on the rachilla, renders it barren; infection at the base of a spikelet renders all the florets of that spikelet barren. These barren florets and spikelets soon become bleached and are conspicuous amongst the green parts of the ear (Pl. XIV, fig. 6). Infection at the base of a spikelet is accompanied by invasion of the rachis, which becomes brown where affected. Under favourable conditions the fungus extends along the rachis, mainly on its exterior, but to some extent internally when the rachis is soft and succulent, and thus other spikelets become affected. Within the bleached pales of barren florets mycelium bearing microconidia is developed even in dry weather.

When the ears were enclosed within glass tubes after inoculation, thus keeping them in a moist atmosphere resembling that of damp weather, the points of infection were more numerous, the bleaching of affected parts more rapid and the extension of the fungus greatly favoured. After five days under these conditions the mycelial growth about the bases of the spikelets and edges of glumes and pales was conspicuous, the mycelium being white, or when bearing the minute sporodochia faintly pink. This feature was observed on ears which had become casually infected in the experimental plot during an exceptionally wet period (1930) (though it was not at all conspicuous), and may therefore occur also in field crops.

Similar methods of inoculation applied at a later stage when the grains were developing in the ears caused infection in similar manner, but the florets finally contained small, shrivelled grains, on which there was frequently a delicate white mycelium or its remains. In many grains the embryo was replaced by a small mass of mycelium, but in most grains, infected later by extension of the parasite or by a different course of invasion, the embryo was present but sometimes diseased. The subsequent growth of such grains is illustrated in Pl. XII, figs. 1 and 2.

Whilst attack on the ears in a field crop frequently follows the lines indicated, another type of attack is equally common. This appears as a single spot on an outer or a flowering glume, the spot having a bleached

centre surrounded by a dark-brown zone. In moist weather these spots are easily seen. The bleached area increases in size as the brown zone progresses outwards. As the ear matures and loses its green colour the bleached areas become scarcely discernible and the brown markings faint and diffuse. This happens also in dry weather, so that in dry weather and with mature ears affected parts are not easily seen. Infection of the flowering glume is followed by penetration and infection of the grain, usually on its anterior side, the latter showing a faint ochraceous colour at the point of infection and a more diffuse brownish-grey colour round about. Infection of single grains in this way is more frequent in barley and oats than in wheat. Although grains may become infected during ripening, field observations indicate that much, perhaps most, of the infection by Gibberella and Fusarium occurs after reaping, when the corn is standing in stooks. Diseased ears, frequently bearing vast numbers of conidia, are then in contact with unaffected ears under conditions very favourable for infection both as regards susceptibility of the ears and growth of the fungus.

The attack on the ears of cereals by *G. Saubinetii* so closely resembles attacks by *F. culmorum* and *F. avenaceum* that it is not possible to distinguish between them by simple observation. The only distinguishing features occur under prolonged moist conditions, when the Fusarium species produce masses of spores known as "mucous mould," whilst Gibberella produces a mycelial growth, either white or faintly pink, the spore masses when present being minute, scarcely discernible even with a hand-lens, and not forming a "mucous mould."

Frequency and geographical distribution in the British Isles.

It is impossible to ascertain by simple observation the frequency of occurrence of *G. Saubinetii* over wide areas in this country, because it is so often associated with species of Fusarium that cause similar types of disease. Laboratory work is essential for recognition of the causal organism. Judging from the number of isolations made for laboratory purposes during the past five years[1], a statement previously made by the writer that "Fusarium disease of wheat, common in the north of England, is due mainly to *F. culmorum* (W.G.Sm.) Sacc. and *F. avenaceum* (Fries.) Sacc., occurring separately or together," has been fully substantiated; and it almost certainly applies to the whole country. Since these two fungi are not only more frequent, but also cause greater damage, they are to be considered the principal fungi concerned in the

[1] A considerable number of species are to be dealt with in due course.

Fusarium-type ("fusariose") of cereal disease. This is contrary to the position in the wheat-growing areas of the U.S.A., where, in 1919, according to Dickson *et al.*(6) "wheat scab (*i.e.* ear blight) was due in 98 per cent. of the cases examined to *G. Saubinetii*, the remaining 2 per cent. being *F. avenaceum*."

G. Saubinetii occurs in all parts of England and has been recorded once in Ireland[1]. The writer has isolated it, and verified its occurrence, from wheat and barley grains and from the bases of wheat, barley, oats, rye, and *Triticum monococcum*, grown in the following areas: Northumberland, Durham, Cumberland, Yorkshire, Cheshire, Staffordshire, Shropshire, the Fens, Cambridgeshire, Essex, and Devon. Mr Dillon Weston, when sending specimens of *T. monococcum*, pointed out that it was considered to be immune from attack by most pathogens; it is not, however, immune from attack by species of the Fusarium group. After examining further samples of seed grain from different parts of the country over a number of years it is hoped to issue a more detailed record of the occurrence of *G. Saubinetii* in Britain.

Thanks are tendered to the Advisory Mycologists and others interested in cereal crops for supplying diseased material from the areas mentioned; to Mr C. Brett for samples of suspected seed from the Seed Testing Station; and to Dr G. H. Pethybridge for unstinted assistance generally.

Summary.

G. Saubinetii (Mont.) Sacc., the conidial stage of which is known as *Fusarium graminearum* Schwabe, produces mature perithecia on natural and artificial substrata, given ample moisture and a uniform temperature of 21° to 22° C. British summer shade temperature does not suffice for this.

The minimum temperature for the germination of conidia and ascospores is about 5° C. Between 5° and 10° C. both kinds of spores develop mycelia, and the fungus can infect cereal seedlings. The optimum temperature for vegetative growth of the fungus and the production of mature perithecia is about 24° C.; the maximum temperature for growth is about 37° C.

In its vegetative and perithecial stages this fungus can withstand prolonged exposure to a temperature range of − 1° to − 20° C. and in its vegetative stage it persists through the winter in this country.

[1] By Mr H. A. Lafferty, in 1918 on wheat, in Galway: an unpublished record seen by the writer in 1930; the description and spore measurements agree with those of *G. Saubinetii*.

Under storage conditions it retains its vitality for at least two years in its vegetative stage, and for at least one year (probably much longer) in its perithecial stage.

Heating to 100° C. for five minutes under dry conditions kills *G. Saubinetii* on or in diseased grains, but this treatment of seed grain is not a practicable way of controlling the disease.

G. Saubinetii causes "seedling blight" and "foot rot" of cereals, but is much less virulent than *F. culmorum* and *F. avenaceum* in this respect. All three fungi attack the ears in a similar way, with similar virulence and with similar results.

G. Saubinetii occurs throughout England and in Ireland, but full details of its frequency of occurrence in the British Isles have not yet been obtained.

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Fig. 1.

Fig. 2.

BENNETT.—*Gibberella Saubinetii* (Mont.) Sacc. on British Cereals (pp. 158–177).

Fig. 3.

Fig. 4.

BENNETT.—*Gibberella Saubinetii* (Mont.) Sacc. on British Cereals (pp. 158–177).

Fig. 5.

Fig. 6.

Fig. 7.

BENNETT.—*Gibberella Saubinetii* (Mont.) Sacc. on British Cereals (pp. 158–177).

EXPLANATION OF PLATES XII—XIV

PLATE XII.

Fig. 1. Wheat from ears (3) not inoculated, (1) inoculated and covered three days, (2) inoculated and covered five days; inoculation with *G. Saubinetii* after grain formation; generally poorer plants but no marked "seedling blight."

Fig. 2. Barley from ears (3) not inoculated, (1) inoculated in the early grain stage and covered three days, (2) inoculated when grains were nearly mature and covered three days; affected grains give seedlings with discoloured bases, and some of them collapse.

PLATE XIII.

Fig. 3. Effect of *G. Saubinetii* on wheat (Little Joss) grown to maturity from artificially contaminated seed in sterilised soil (left), and from healthy seed in contaminated soil (right). Result, fewer ears, poorer grain, more non-flowering tillers, but no failure to extrude ears and no "whiteheads" with "deaf" ears.

Fig. 4. Effect of *G. Saubinetii* on barley (Plumage Archer) under the same conditions as wheat (above). Marked reduction in growth, with thin, weak stems, especially in contaminated soil (right) where roots suffered seriously.

PLATE XIV.

Fig. 5. Effect of *G. Saubinetii* on oats (Swedish Crown) under the same conditions as wheat and barley (above). Reduction in vigour, especially in contaminated soil (right), and increase in the number of barren spikelets.

Fig. 6. Wheat ears; left, several casual infections by *G. Saubinetii*; right, barren florets, spikelet, and whole ear, following artificial inoculation with *G. Saubinetii* at flowering time.

Fig. 7. Barley ears, inoculated with *G. Saubinetii* on emergence from the sheath. Result, all the lower spikelets bleached and barren, whilst some upper spikelets bear small diseased grains.

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A NEW BUNT ON WHEAT IN INDIA

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(With Plate XV and 1 Text-figure.)

Specimens of diseased seeds of eight hybrid wheats were collected at Karnal, Punjab. These wheats were from crosses between Federation and Pusa 4 and 52, respectively, and had been bred in the Botanical section at Pusa and sent to Karnal for trial under Punjab conditions[1]. Examination showed the presence of a species of *Tilletia* which appeared to differ from *Tilletia tritici* (*Caries*) and *T. laevis* (*foetens*). These latter have been previously recorded on wheat. This determination was confirmed at the Imperial Bureau of Mycology and the name *Tilletia indica* n.sp. has been proposed for the new smut. This smut affects only partially the kernels which are not swollen. The embryo tissue is not destroyed by the smut and in a large number of cases only the embryo portion is infected. In some cases the infection spreads to the tissues along the groove, but the endosperm material lying along the smooth side of the grain is uninfected; thus this fungus is more highly differential than *T. tritici* (*Caries*) and *T. laevis* (*foetens*) which leave only the glumes and epidermal tissues of the kernels unaffected. Text-fig. 1 shows the various stages of infection and Plate XV indicates spore shape and the manner of attachment. The following is the diagnosis.

Tilletia indica *n.sp.*

Sori in ovaries form dusty spore masses, oblong or ovoid, 1–3 mm. in length, brown to dark brown, partially destroying the kernel, attack starting at the hilum and running along the groove, leaving the endosperm intact covered by the whole or partially ruptured seed-coat.

Spores when mature are brown to dark brown, spherical or subspherical or oval, 22–42 × 25–40 μ in diameter, average 35·5 μ and mode at 36 and some spores may reach a length of 55 μ. The proliferations of the epispore are reticulate, the ridges or scales somewhat roundish or irregular showing at the circumference of the spore a band about 2–4 μ in breadth. Mixed with the spores are numerous large yellowish or sub-hyaline sterile cells, rounded or angular and smaller in size than the

[1] Some of these wheats were grown in Pusa and so far no such disease has appeared.

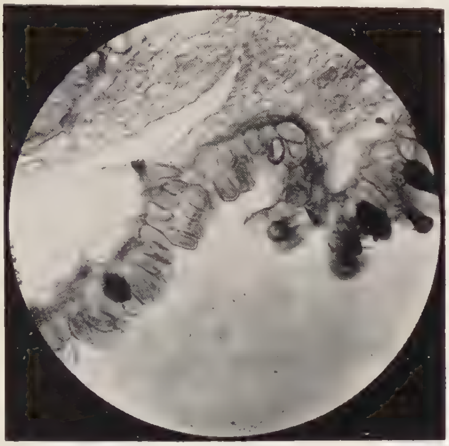

Fig. 1.

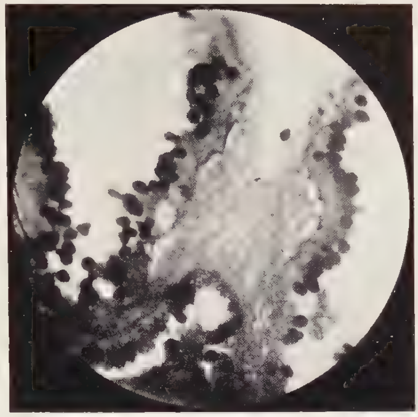

Fig. 2.

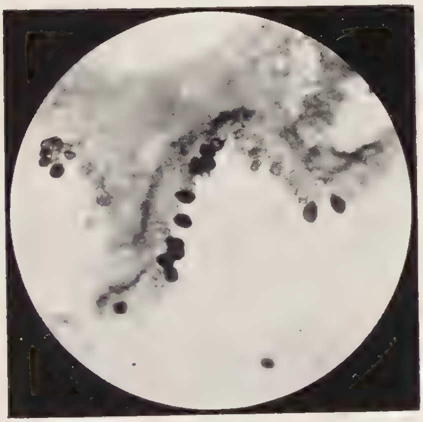

Fig. 3.

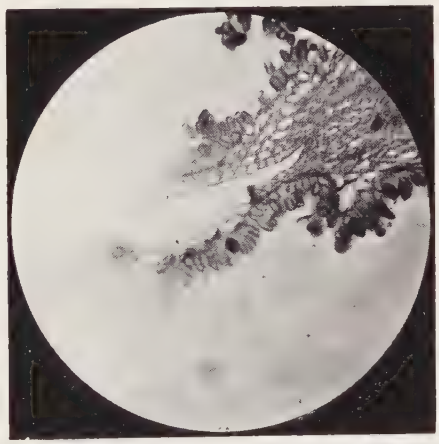

Fig. 4.

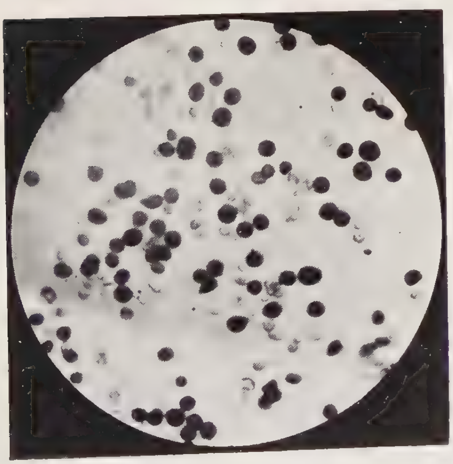

Fig. 5.

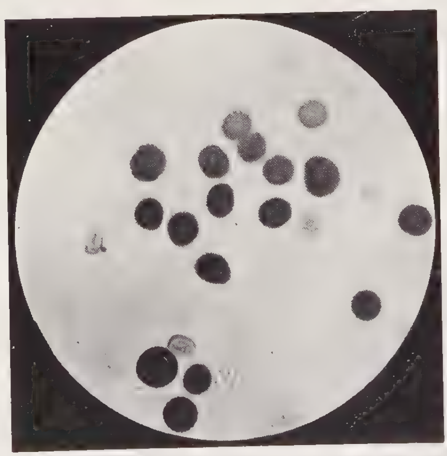

Fig. 6.

MITRA.—A New Bunt on Wheat in India (pp. 178–179).

spores, with comparatively thinner walls. These correspond in part to undeveloped spores. The spores often have a papilla or thread of detachment at one side.

On *Triticum vulgare*, India.

Text-fig. 1. Diseased wheat grains showing various stages of infection with *T. indica* n.sp. ×6.

This species of *Tilletia* is similar to *T. tritici* (*Caries*), but unlike the latter it has no greasy spore mass and no smell of rotten fish and the spore mass dries into pustules which are capable of being blown about by wind. It resembles *T. tritici* in having reticulate walls and differs from it in having much larger spores and also in its effect upon the kernels. It does not resemble *T. laevis* (*foetens*).

Attempts to germinate the spores have failed.

EXPLANATION OF PLATE XV

Microscopic photographs of sections of wheat grains showing the infected regions, spore shape, etc. ×500.

(*Received December 2nd*, 1930.)

STUDIES IN BACTERIOSIS

XIX. RESEARCHES ON THE GROUP OF GREEN-FLUORESCENT BACTERIA, PART I; *BACTERIUM TRIFOLIORUM* (JONES *ET AL.*) AS THE CAUSE OF A DISEASE OF *VICIA FABA*

By MARGARET S. LACEY.

(*From the Bacteriological Laboratory of the Imperial College, South Kensington, London.*)

(With Plate XVI.)

During the last few years, diseases of a wide variety of plants have been attributed to bacteria of the green-fluorescent group by numerous investigators, who, either on account of somewhat small cultural distinctions or from differences in pathogenicity on various host plants, in most cases regard their particular strain as a new species. Burkholder (1) in a recent paper on the genus *Phytomonas*[1], draws attention to the confusion arising from this tendency to create new species. He says "too many species have been described on the basis of pathogenicity to some host, with a few cultural and morphological characteristics which are slightly more than sufficient to place them in a genus. Work of this type simply lays foundations for synonyms." Burkholder lists 18 species, pathogenic to plants, which produce a green-fluorescent pigment on beef extract agar, but states that there are others not brought out in his tables, as "it is known that certain of the green-fluorescent types will not produce pigment on beef-extract agar. Furthermore, this ability to produce pigment is frequently lost in pure culture. For these two reasons it is felt that a number of species belonging to this group may be found with the white or colourless forms." A study of Burkholder's tables reveals the presence of 8 species in his "colourless" group which in the original description are stated to produce green-fluorescent pigment on certain media, and in addition, descriptions of 7 other species have been found in the literature which are not included in his tables. As these 33 species all show close similarity in cultural and physiological behaviour the identification of a freshly isolated strain belonging to this

[1] Bergey's classification (Bergey's *Manual of Determinative Bacteriology*, Williams and Wilkins, 1923).

group is not easy, while the determination of the rôle played by such an organism in the production of certain plant lesions is rendered more difficult by the fact that both *B. fluorescens liquefaciens* and *B. fluorescens non-liquefaciens* (saprophytic strains which closely resemble the pathogenic species) often follow rapidly on a primary infection by another organism and develop to such an extent that they frequently crowd out and supersede the original producer of the disease. During the course of several years' work on bacterial diseases of plants, organisms belonging to the green-fluorescent group of bacteria have been obtained from various plant lesions on numerous occasions. In some cases (such as that of parsnip roots with internal necrotic lesions and of certain diseased potato tubers) it remained open to doubt whether the green-fluorescent organisms isolated from the lesions were indeed the primary cause of the disease. In such cases the cultures when first isolated exhibited a feeble virulence which was quickly lost and it was considered probable that the strains were those of saprophytic species which had attained a passing pathogenicity by *passage* through a host rendered susceptible by some primary cause, such as exposure to frost. In other cases the isolations were made from plants suffering from known diseases and the cultures obtained from the lesions were found to agree with the original descriptions of the causal organisms, for example *B. delphinii* from Delphinium and *B. trifoliorum* from a leaf spot of clover. The similarity between all these strains was soon noticed and from time to time cultural comparisons were made and cross-inoculations on the various host plants were attempted, but without any definite proof of the inter-relationship between them being established. During the spring and autumn of 1929, however, specimens of badly diseased broad bean and lettuce plants, potato tubers, and seeds of *Medicago lupulina* were received for examination, from all of which strongly virulent cultures of organisms of the green-fluorescent group were obtained. These strains, although showing cultural differences sufficient (according to the present method of classification) to warrant their separation into distinct species, were each pathogenic to broad beans, lettuce and potato tubers, on all of which the lesions produced were the same for each organism. Further, when attempts were made to identify the strains, each was found to correspond closely to several different pathogenic species and some positive results were obtained by inoculation of the new strains on to certain of the host plants attacked by these species.

This series of papers deals with the identity of these strains and gives the results obtained by cultural comparisons and inoculation experiments

carried out with these and certain other strains. The results indicate that there is a close relationship between certain species of the green-fluorescent group in pathogenicity as well as in cultural reactions, and suggest that certain diseases may be caused by several slightly different strains rather than by one fixed species. Comparisons are also made between the pathogenic species and the nearly allied saprophytes *B. fluorescens liquefaciens* and *B. fluorescens non-liquefaciens*, and various attempts to induce the latter to become pathogenic are described.

The present paper gives an account of the disease of *Vicia faba* mentioned above, and of work done on the identification of the causal organism with *B. trifoliorum*.

Description of the disease.

In May 1929 a severe outbreak of disease occurred among the plants in a field of *Vicia faba*. When first examined, many of the plants were in a very bad condition, the apical buds being reduced to a black slimy mush, from which the infection was travelling rapidly down the stems, the lower parts of which were quite sound. In less severely attacked plants the young leaves bore large black necrotic areas, and sometimes the lesion had dropped out, giving a shot-hole effect. This was particularly marked round the leaf margins, and at first the impression was gained that the leaves had been severely gnawed by some insect, but exactly the same effect was produced later by inoculation with the causal organism.

In other cases the stems were of a jet black colour and were severely rotted, the trouble apparently starting at the ground level and proceeding upwards, suggesting soil infection.

Isolation of a causal organism (strain 212).

From the most recently attacked tissue isolation plates were made on which numerous very virulent colonies developed. Several of the colonies were obtained in pure culture, and were found to be morphologically and physiologically identical, and all were very virulent for *Vicia faba*, producing lesions, both on stems and young leaves, similar to the original disease (Plate XVI, fig. 1). Re-isolations from these lesions invariably yielded large numbers of the same pathogen.

Summary of inoculations of *Vicia faba* with strain 212.

Rapid infections of stems and leaf-buds were obtained at temperatures ranging from 16° to 27° C., the severity of the attack increasing with increasing temperature. After 24 hours at 16°–20° C., stems inocu-

lated by a prick of a needle smeared with a culture of the organism were blackened and rotted for 1–2 inches, while at temperatures above 20° C. the greater part of the stem became involved and a black fluid swarming with bacteria oozed from it. In some cases when the parts above and below the blackened area appeared sound externally, the vascular bundles were blackened for a considerable distance in both directions, and the vessels were filled with bacteria. Bud infections, like those on the stems, became more severe with increasing temperature, large, black, rotted areas developing on the young leaves in 24–48 hours after inoculation either by needle pricks or by drops of bacterial emulsion placed on the uninjured surface. In one case the veins of a leaf became infected from a petiole inoculation and showed jet black against the green mesophyll tissue (see Plate XVI, fig. 1). On the other hand, several attempts to produce lesions on the older leaves resulted in complete failure. Below 16° C. there was but little infection. At a temperature varying from 14° to 17° C. local blackening and rotting round the inoculation point only occurred, and inoculations below 14° C. gave a negative result. Most of these inoculations were made on plants kept in a moist chamber for 24–28 hours after inoculation, but just as severe infections were obtained on plants in the open, left uncovered, when the inoculations were made during periods of dull and showery weather. In July, 1929, however, during a period of drought, inoculation of buds of plants grown in the open failed, while stem inoculations resulted in the formation of black sunken lesions, about 2 inches long, round the points of inoculation, but these dried out later and the plants outgrew the disease. A humid atmosphere is therefore necessary for the full development of the disease.

DESCRIPTIONS OF STRAINS 212.

A short rod, motile by means of 1–6 polar flagella; aerobic; gram negative; no spores; optimum temperature 30° C.; no growth at 37° C. No liquefaction of gelatine; no production of indol; diastatic action weak or absent; no reduction of nitrate. Acid is produced from dextrose, galactose and feebly from saccharose, none from lactose, mannite, maltose, dulcite, sorbite, salicin, inulin or raffinose. Milk becomes strongly alkaline, no coagulation or digestion, no reduction of the litmus. Good growth in Fermi's and Uschinsky's solutions, with formation of a thick mucoid yellow pellicle and yellow-green fluorescence. Green colour also produced in gelatine media, but not in bouillon or beef-extract agar, p_H 6·8–7·0, the latter medium becomes brown. Growth on bouillon agar

yellowish-white; in old cultures the thickest part of the growth frequently becomes yellow or even brown. Colonies on bouillon-agar plates are extremely variable, exhibiting all stages from translucent, smooth, round, slightly convex colonies with entire or slightly crenated margins, to a transparent flat growth spreading in a thin film over the surface. Each type of colony can give rise to the others on replating, and all were identical in respect of virulence and cultural reactions.

Comparison of strain 212 with *B. trifoliorum*.

In August, 1928, an organism (strain 202), identified as *B. trifoliorum*, had been isolated from a leaf-spot disease of red clover, but no inoculation experiments had been made at the time. The similarity of strain 212 to this clover strain being observed, a series of cultural comparisons were made and the two strains were found to be practically identical in cultural characters, while on inoculation into broad-bean plants the clover strain 202 produced similar lesions to those caused by strain 212 but was less virulent (Plate XVI, fig. 2). Plants of red clover were then inoculated by spraying with the two cultures. Some of the leaves infected with the bean strain 212 developed numerous small reddish brown spots on both surfaces, and a few similar spots also occurred on the leaves infected with strain 202. (The clover strain, having been kept on artificial media for a year, had doubtless lost some of its virulence.) The control plants remained free from leaf-spots.

Comparison of *B. trifoliorum* (strains 202 and 212) with other plant pathogens of the green-fluorescent group.

In addition to *B. trifoliorum* five other plant pathogens, namely *B. glycineum*, *B. medicaginis*, *B. medicaginis* var. *phaseolicola*, *B. nectarophilum* and *B. lacrymans*, all non-liquefiers of gelatine, appear to be closely related to strains 202 and 212. The first of these, *B. glycineum*, was described by Coerper (3) as causing angular leaf-spot of soy bean. Coerper found that considerable variation might occur among the colonies of *B. glycineum* developing on the same plate and that one strain caused a browning of bouillon-agar medium, thus in both these characters, as well as in other physiological reactions, resembling strain 212. Jones (4), however, obtained negative results by inoculation of *B. trifoliorum* on soy beans. *B. trifoliorum* also failed to attack alfalfa, the host plant of *B. medicaginis*, which differs culturally from the former species in not producing acid from glucose or saccharose.

B. medicaginis var. *phaseolicola* is culturally very similar to strains

202 and 212, but this species, which is pathogenic to *Phaseolus vulgaris*, *P. multiflorus* and *P. lunatus*, was found by Burkholder (2) to be avirulent to certain plants including soy bean, red clover and broad beans. The fourth species, *B. nectarophilum* (Doidge), the cause of pear-blossom blight in S. Africa, is similar to strains 202 and 212, except that it produces no acid in saccharose broth. This character, however, appears to be of little diagnostic value in the green-fluorescent group, in which the fermentation of saccharose is never strong, and may vary even between strains isolated from the same lesion. For example, two strains from colonies on an isolation plate from a leaf-spot of larkspur seedlings proved identical in cultural characteristics, and agreed with *B. delphinii*, with the exception that while one strain produced acid in saccharose broth the other did not give the least trace of acid in several tests. Also, this character may vary in a single culture, for although a definite acid reaction was obtained with every colony of strain 212 on first isolation, the power to produce acid in saccharose broth had been completely lost after eighteen months on artificial media although the cultures were still virulent and unaltered in every other respect.

Finally, *B. lacrymans*, producing angular leaf-spot of cucumber, appears to be closely allied to *B. trifoliorum*.

In addition to these plant pathogens two saprophytic species, *B. fluorescens non-liquefaciens* and *B. striata* (found in soil), show close cultural agreement with strains 202 and 212, but inoculations of *Vicia faba* with cultures of the former species invariably gave negative results. The resemblance of these saprophytic strains to the pathogenic species is, however, so marked as to raise once again the question, suggested by various authors, whether all the pathogens of the green-fluorescent group are not parasitic strains of *B. fluorescens liquefaciens* or *B. fluorescens non-liquefaciens*.

Further cultural comparisons and inoculation experiments with strains 202 and 212 will be given in later papers.

In conclusion, I wish to express my thanks to Dr S. G. Paine for his advice and criticism throughout this investigation.

SUMMARY.

A disease of *Vicia faba* is described and the causal organism identified as *B. trifoliorum*. This species is compared with other members of the green-fluorescent group of bacteria.

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(1) Burkholder, W. H. (1930). The genus *Phytomonas*. *Phytopath.* xx, 1.

(2) —— (1926). A new bacterial disease of the bean. *Ibid.* xvi, 915.

(3) Coerper, F. M. (1919). Bacterial blight of soy bean. *Journ. Agric. Res.* xviii, 179.

(4) Jones, L. R., Williamson, M. M., Wolf, F. A. and McCulloch, L. (1923). Bacterial leaf-spot of clovers. *Ibid.* xxv, 471.

EXPLANATION OF PLATE XVI

Fig. 1. Broad-bean plant inoculated with strain 212, 24 hours at 80° F. Showing invasion of the veins of a leaf from a petiole infection, also severe stem lesions and black spot on a young unfolded leaf.

Fig. 2. Broad-bean plants inoculated with strains 212 (broad bean) and 202 (clover) respectively. Photograph taken after 24 hours at 64° F.

(*Received November* 13*th*, 1930.)

Fig. 2.

Fig. 1.

LACEY.—STUDIES IN BACTERIOSIS (pp. 180–186).

YIELD STUDIES IN OATS

IV THE INFLUENCE OF CLIMATIC FACTORS UPON THE GROWTH OF A SPRING SOWN VARIETY "RECORD"

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(With 3 Graphs.)

Introduction.

During the last decade methods of analysis of growth rates have been suggested by numerous workers, by means of which it has been thought possible to study to a limited extent the influence of the various factors constituting "climate" upon the growth of plants. The earlier works of Briggs, Kidd and West (7) in which they employed the data of Kreusler concerned with the development of maize, dealt also with the methods of calculating the indices of growth used. Brenchley (4) has correlated the growth of peas with the climatic factors and also studied the influence of certain factors upon the rate of growth of barley (5); Inamdar, Singh and Pande (15) have investigated the growth rates of cotton, and Vyvyan (18) has paid more particular attention to the rate of leaf development. Alun Roberts (1), by the analysis of the yields of oats over a period of many years, has been able to trace the effects of some of the climatic factors, whilst the work of Fisher (10) on the relation of rainfall and the yield of wheat has also attracted attention to the method of correlation studies as a tool to be used in such agronomic investigations. Gregory (13) has perhaps made the most searching analysis upon such lines; he worked with barley.

In connection with other studies made with oats in close co-operation with the geneticists of the Station, it appeared that some knowledge of the effect of climate upon the rate of growth was desirable; the growth of our common field crops must be studied before we can arrive at a complete understanding of the yield problem with which the geneticists are concerned. When the experiments were started there was a lack of experiments of a field nature, for the plants of Kreusler (7) were well spaced apart, those of Singh (15) and Gregory (13) were grown in pots—

the results obtained by Gregory were not then available. The reason for this dearth of experimental data is not far to seek, for, as Blackman (3) has pointed out recently, the work is somewhat laborious, as many plants have to be dealt with in order to overcome the difficulties caused by plant-to-plant variation and soil heterogeneity. Our experiments were designed in the hope that by a careful study of the rate of growth for several years it would be possible to arrive at a solution of the question—"Is there a master factor?" and in the hope that it would be possible to estimate, to a limited degree, the influence of the factors of the weather in terms of the ordinary meteorological data. Unfortunately, changes in the personnel of the Station staff prevented this work from being continued for more than two years, but the results obtained are reported as they may prove of value to others who desire to test the methods with field crops.

A. Methods of collecting primary data.

Having decided to employ the ordinary method of planting as against the method of using pot cultures or spaced plants, it will be realised that the factor of competition is thereby introduced; a competition between roots and also between shoots which may perhaps mask or obscure the effect of some of the climatic factors or give unusual prominence to the part played by others in the growth of the plants, as contrasted with the effect similar factors would have upon free-growing plants.

The plot of ground selected for its believed homogeneity was situated at the Welsh Plant Breeding Station Farm at an elevation of approximately 500 ft. above sea level. The plants were exposed to the full rigour of the climate, enjoying no shelter of any kind from the dates of sowing, on March and April 19th of each year. The carefully graded seed was shown in drills spaced 12 in. apart, the rate of sowing was designed to give twenty established plants to the foot in the drill; this number was obtained. Samples were taken at weekly intervals, the total length taken being nearly 100 ft. of drill; after an inspection of all the rod-rows the damaged plants or any particularly large or small plants at the *end* of rows were marked and discarded. From four or more rows the plants were pulled up by hand, counted and then the roots were carefully cut off. From another row (or rows) ten short lengths of 1 ft. (approx.) were taken by digging the plants up and washing the soil away. For the dry weight estimations, at least 600 plants were weighed in batches of twenty; for leaf-area determinations it was customary to employ samples of sixty plants, as tests especially designed to indicate the degree

of accuracy to be expected from the methods had shown these numbers to be on the whole satisfactory. This was confirmed by the data of the experiments themselves which gave errors (standard deviation) always less than 4 per cent. of the reading of the average dry weight, and leaf area, where a part of the error lay in the method of estimation itself.

As some 30,000 plants were dealt with in a season it was necessary to complete the drying and weighing of one weekly "harvest" before the plants of another had to be dealt with; the plants were dried at 85°–90° C. in an oven.

The meteorological data were obtained from the observational station situated 100 yards away.

B. The general growth of the plants.

The first date of sowing, March 19th, was rather late, but it was chosen as being a date on which it is usually possible to carry out the operation, without being a date so far removed from practice as to make the experiment very unusual in this respect. The second date of sowing, a month later, was chosen as being likely to provide an opportunity of observing young plants under warmer conditions than those enjoyed by the first series. It must be remembered that a date had to be selected that would be practical from the point of view of previous cultural operations also.

The soil conditions that deserve mention are that, generally speaking, one would describe the soil as a light loam, small stones being present; it is a shallow soil overlying a shale rock and one upon which even very heavy rain does not cause the water to lie in small pools for any length of time. Previous cultivation had been concerned chiefly with pedigree grasses; a small dressing of superphosphate had been given a year earlier. As the plots selected each year were similar, it will suffice to show the general nature of the soil by quoting the analyses made by the Chemical Department:

Mechanical composition:	%
Fine gravel	13·8
Coarse sand	11·6
Fine sand	12·7
Silt	13·3
Fine silt	25·1
Clay	8·8
Hygroscopic moisture	4·01
Loss on ignition	11·66

Chemical composition:

	%
Nitrogen	0·37
Calcium oxide (CaO)	0·22
Magnesium oxide (MgO)	0·49
Phosphoric acid total (P_2O_5)	0·25
,, ,, available	0·014
Potash total (K_2O)	0·46
,, available	0·016

"The soil is a thin, light loam, formed from the Aberystwyth grits, a sub-group of the lower Silurian. Like other soils in the neighbourhood, it contains no carbonate of lime."

Seven weeks after sowing the leaves were about 5 sq. cm. in area and the plants, when dried, weighed 2 or 3 gm. per 100. At this early stage the first estimations were made. The maximum leaf area was attained approximately three months after sowing, whilst the maximum dry weight was reached (in the first sown series) in five months' time. No determinations were carried out after mid-August with these series. On the whole the plants were singularly free from pests and disease when sown at the earlier date, but some mildew appeared on the later-sown series. In making leaf-area determinations, only clean, green laminae were estimated, all discoloured or naturally withered portions (as well as those plants covered by any fungus hyphae) were discarded. No diseased plants were utilised for calculating the growth indices. Small dead tillers were weighed, which means that in the later stages of growth only a small part was lost owing to the partial decay of these tissues. Such a loss, quite unavoidable, is not serious when the plants are grown close together, for the branching is restricted. The leaf "lamina" only was estimated as "leaf area," the sheath being thus excluded.

C 1. The primary data and the derived indices of growth.

A sample of the primary data is here quoted in order that the reader may be better enabled to follow the different processes.

Table I.

Date of lifting	No. of plants	Average dry weight per 100 gm. "W"	Average leaf area sq. cm. "L"
25. v. 27	817	$4·37 \pm 0·08$	$11·6 \pm 0·25$
1. vi. 27	757	$8·07 \pm 0·31$	$17·51 \pm 0·50$
8. vi. 27	746	$18·93 \pm 0·34$	$43·00 \pm 0·97$

The relative growth rate was calculated by the formula

$$\log_e W_2 - \log_e W_1 = \frac{R}{100},$$

that is to say, by subtracting the Napierian logarithms of the dry weights obtained at the two dates. A similar formula underlies the method of Blackman (2) in which growth is considered as a process of compound interest (the rate of "interest" is not considered as necessarily constant). In precisely the same way the rate of the growth of the leaves was calculated. To obtain the mean leaf area between two dates the formula

$$\frac{L_2 - L_1}{\log_e L_2 - \log_e L_1} = L,$$

was employed. This is the method recommended by Fisher (11) as likely to be the more accurate whatever the relation between time and the leaf development.

If we now divide the increase in dry weight by the leaf area so obtained we have the "Unit Leaf Rate" of Briggs, Kidd and West (7), or the "Net Assimilation Rate" of Gregory (13). Merely from a point of view of interest, the linear calculations of the "Unit Leaf Rate" and "Relative Growth Rates" were also made, and it was somewhat surprising to find that the two methods gave results in very close agreement, despite the criticisms levelled at the linear method. As Fisher (11) points out, the discrepancies are often due to the false mean selected.

These then are the primary data and derived indices of growth.

C 2. THE RELATIVE GROWTH RATE "R" OR "THE EFFICIENCY INDEX."

The values of this index for each series of plants were plotted against the time from the date of sowing, and it was seen that the four curves bore a general resemblance to each other; the value of "R" rises to a maximum very early in the life cycle and then falls, with fluctuations, to zero. If the magnitude of this index was *primarily* determined by the climatic factors then the values for the two series growing side by side should show parallel fluctuations; these were not observed. If, on the other hand, the value of this index depends upon the "physiological-age" of the plants, then there may exist between plants of the same "time-age" a general similarity in their rates; this was observed.

If for the moment we assume that the relation between the values of "R" and time will be such as to allow us to correlate the values of "R" with one another on a basis of age so calculated, the following coefficients are obtained:

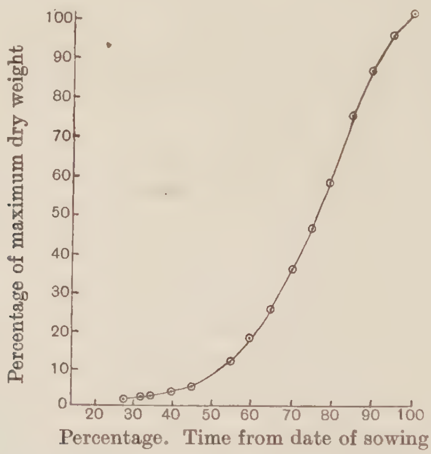
1926.	"R_1" and "R_2,"	plants of the same time-age,	0·743 ($P<0·01$)
1927.	"R_1" and "R_2,"	,, ,,	0·685 ($P<0·01$)
1926 and 1927.	"R_1" and "R_1,"	,, ,,	0·587 ($P<0·01$)
,,	"R_2" and "R_2,"	,, ,,	0·640 ($P<0·01$)

where "R_1" and "R_2" are the values of the index for the two series of plants sown at an interval of four weeks.

There is no such resemblance between the fluctuations of the values of "R" for the two series of the one year over the same period of growth.

1926. "R_1" and "R_2," same period of growth, 0·280 ($P>0·1$)
1927. "R_1" and "R_2," ,, ,, 0·151 ($P>0·1$)

All these considerations, and those of the graphs themselves, point to the conclusion that the value of the index "R" is dependent on the "time-age" of the plant and/or the stage of growth already attained, *i.e.* the "physiological-age," and that the climatic factors may modify the values for the period. It is therefore necessary to try to obtain some



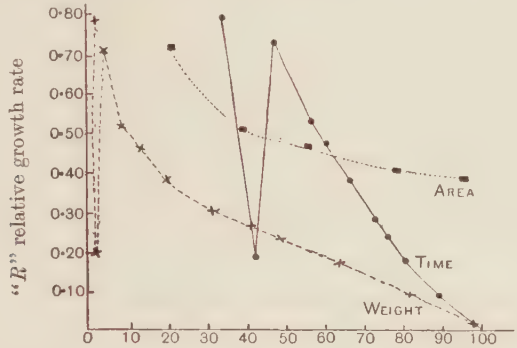
Graph 1. The mean curve of the increase in dry weight of the "tops" of Record oats (spring sown) from the time of sowing to the time of maximum dry weight.

approximation to the mean drift with time of the value of this index before we can determine the influence of the climatic factors upon the rate observed during any period.

(The methods of Gregory have been followed in general outline; these, and the assumptions involved, have been criticised by Briggs and a reply by Gregory has also been published; we will refer to these considerations later.)

In order to obtain a mean curve for the rate at which the dry matter accumulated, the data of the four series were all re-calculated on a percentage basis, the percentage of the maximum dry weight attained being plotted against the percentage of the growth period. As the growth periods were of almost equal length, this caused relatively little re-

arrangement. From these four curves the mean curve was plotted by calculating mean values (geometric means) at intervals of 5 per cent. along the time scale. This mean curve, an approximation to the "normal" curve, is surprisingly regular when it is considered that only four readings were available. The unsmoothed curve is shown as Graph 1. By reading the values on this curve at the time intervals corresponding to those upon which the primary data were collected, it was possible to obtain a series of percentages of the maximum dry weight. By converting these percentages into dry weights again the dry weight that would have been attained according to the mean rate of growth was found. For each date of estimating the plants there is now an observed and calculated dry



Graph 2. Showing the relationship between the relative growth rate (or efficiency index) and the dry weight of the tops, the leaf area, and the time; expressed as percentages of the maximum dry weight, maximum leaf area and the total period during which the plants of spring sown "Record" oats gained in weight.

weight reading, from both of which the relative growth rates can be found by simply subtracting the Napierian logarithms. The comparison of these two values "R_O" and "R_C" enables us to see whether the rate of growth was above or below the mean rate.

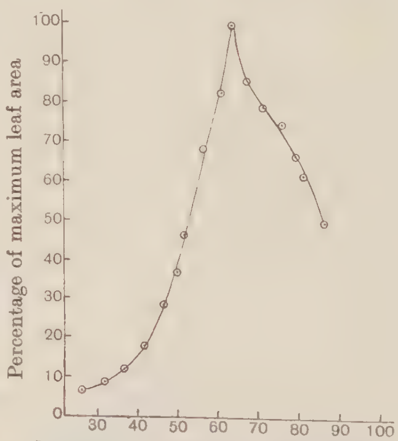
The relative growth rate "R" for the mean curve of increase was calculated, and plotted in turn against time, the weight already attained, and the leaf area developed at the time. (The leaf area used was the mean area, and was calculated similarly to the mean dry-weight increase.) The graphs so obtained are shown as Graph 2. The graph of the value of "R" against the weight already attained, shows a curve which is of two parts; the value of the index "R" decreases at almost a constant rate during the later portion of the period. A very similar curve was obtained by Gregory, but with his pot cultures this linear relationship

did not commence as early as in our field experiments; one tentative explanation offered is that the competition between the plants was responsible for this early decline in the relative growth rate, for as Brenchley (5) has shown with barley, the rate of growth so measured decreases with the progressive decrease in the available food supply for each plant caused by crowding the plants together. Further, the peak of the curve was reached at the time when the plants were just sufficiently large to have "filled up the rows" and were visibly causing mutual interference with development above ground. This can surely be no mere chance coincidence. The graph, "R" against the leaf area, is generally similar, as is that also against time. The former shows a decline from the maximum rate obtained when the plants had developed one-third of their total leaf area.

Reviewing the three curves, it is seen that in a considerable part of the entire life cycle the relative growth rate is almost proportional to the growth yet to be made, so that the usual logarithm formula could be applied as used by Vyvyan with *Phaseolus* leaves.

C 3. The development of the leaf area.

A mean curve of the leaf development was obtained by a similar method to that described for the dry-weight increase. The unsmoothed curve so obtained is shown as Graph 3. It is seen that the maximum area is developed when the plants have been growing for about two-thirds of the total time: during this period they have attained to one-third of their total weight. The leaf structure is formed comparatively early; the time at which the dry weight rises most rapidly is during the period immediately following that of maximum leaf development. The fall in leaf area is a rapid one. It is tentatively suggested that the initial rapid fall in area is due, in part, to the death of some of the side tillers; the subsequent rate of decrease in area is slower. The large fluctuations caused by irregular withering and discoloration observed after



Graph 3. Showing the mean curve of the development of the leaf area of "Record" oats (spring sown) from the date of sowing to that of maximum dry weight.

85 per cent. of the time had elapsed prevented any reliable average from being obtained, the graph is therefore left incomplete. In comparing the rate of increase of the area with that of the dry weight, it must be remembered that only the tops were estimated; provided the leaves do not change greatly in thickness, etc., the development of the sheath and the stem can be traced by a comparison of the two rates—total dry matter of the tops and area.

The ratio $\frac{\text{percentage of maximum weight}}{\text{percentage of maximum area}}$ does not rise sharply from the time at which 35 per cent. dry weight is attained to that of 60 per cent. It is only after the maximum area has been formed that this ratio rises rapidly. Granted the premises concerning thickness, the conclusion is therefore reached that during this 35 per cent.–60 per cent. period the relative amount of food utilised in the production of "stem" dry weight as compared to "leaf" dry weight is almost constant; afterwards the food accumulated is chiefly utilised in stem and flower development. These two periods of growth, the vegetative and the flowering (including the necessary preliminaries of stem formation), stand clearly contrasted. With other gramineae these considerations are of much economic significance, the question of the relative merits of strains may centre around the ratio of leaf/stem at various seasons.

C 4. THE RATE OF GROWTH PER UNIT LENGTH OF DRILL.

In a field experiment of this nature it was necessary to be certain that competition did not kill out many plants at any one stage, and so vary the amount of space available for the surviving plants. To check this and the rate of sowing, the ten short rows were taken each week. During one season these rows were removed with the utmost care possible under the field conditions and the roots subsequently washed free from the soil. Despite this the proportion of "root" to "top" showed a steady decline, believed to be partly due to the greater proportion of root lost in each succeeding week. For this and other reasons, it was not possible to employ the data so obtained to convert weights of "tops" into "totals." These determinations gave satisfaction from the other point of view, namely, the number of plants surviving was practically constant throughout. For this reason the graph of the mean rate of increase in dry weight per foot of row was very similar to that calculated on a basis of a given number of plants. So that the rate of development per unit of ground need not concern us further.

D. The climatic factors of the environment and the plant growth.

Under the conditions resulting from the late sowing the plants were not subject to prolonged low temperature, and the average mid temperature during the period of growth was 55° F. During the period of growth the average weekly rainfall was 1·03 in. Near the coast the westerly winds, or south-westerly winds, are often accompanied by rain, whilst the easterly winds from the hills are relatively dry. During the period of growth of the two seasons there was a positive correlation between the rainfall and the mid temperature, but there was no correlation between the hours of sun and the temperature over the entire period. During the later months of July and August a small positive coefficient was observed. Because of the climatic correlations partial coefficients are necessary when the growth of the plant is considered.

(*a*) *"Unit leaf rate"—"E"—and climatic factors.*

The data in these experiments are concerned with the top growth only. The values of the indices therefore are smaller than those of other workers. With maize, Briggs, Kidd and West dealt with a range of from 3 to 12 mg. per sq. cm.; Gregory does not present his data in such a way that the range of the index is seen readily. In an earlier publication reporting experiments with oats it was observed under greenhouse conditions that at the sixth week of development a value of 3·5 mg. per sq. cm. was obtained. In the present series the values at the seventh week from sowing were all very low; after this date until the time of maximum leaf development the value fluctuated from 0·5 to 5·1; beyond this stage, when the leaf area was decreasing, the fluctuations were wider, but no steady decrease in the unit leaf rate with age was observed before these observations concluded.

The following coefficients were obtained between the unit leaf rate and the climatic factors:

Table II.

Season	Factors	Coefficient	*Probability
1926 and 1927	"E_1" and rainfall† "r"	0·446	$P>0{\cdot}01<0{\cdot}02$
1926 and 1927	"E_2" ,, ,,	0·330	$P=0{\cdot}02$
1926 and 1927	"E_1" ,, mid temperature "mT"	0·659	$P<0{\cdot}01$
1926 and 1927	"E_2" ,, ,, ,,	0·617	$P<0{\cdot}01$
1926 and 1927	"E_1" ,, max. ,, "MT"	0·589	$P<0{\cdot}01$
1926 and 1927	"E_2" ,, ,, ,,	0·426	$P>0{\cdot}01<0{\cdot}02$
1926 and 1927	"E_1" ,, min. ,, "mt"	0·515	
1926 and 1927	"E_1" ,, hours of sunshine "S"	0·055	$P>0{\cdot}1$
1926 and 1927	"E_2" ,, ,, ,,	0·180	$P>0{\cdot}1$

* See Fisher (12). † Previous rainfall, see p. 197.

Whereas the coefficients of correlation between the values of this index "E" for plants of the same age but sown at different dates were not significant, a positive and significant coefficient was found between the values of the two series of plants growing for the same period and so enjoying the same meteorological conditions. The inference is therefore that the magnitude of this index is controlled by these conditions.

The following partial coefficients were obtained by eliminating the various climatic factors:

"E" and mid temperature ...	. rain and sunshine	0·706 ...	signif.
"E" and rainfall ...	. mid temp. and sunshine	0·559 ...	signif.
"E" and sunshine ...	. mid temp. and rain	0·270 ...	insignif.

For these partial coefficients the entire series (both early and later sowings) were used.

It is seen therefore that the unit leaf rate is most closely correlated with the temperature conditions; the influence of rainfall is also clearly seen. The solar radiation, expressed as hours of sunshine, is considered as without effect, as the coefficient obtained is not significant. The influence of temperature upon the unit leaf rate of maize and barley was similarly observed by Briggs, Kidd and West and by Gregory, although their coefficients were not so large. With maize the coefficient obtained with rainfall was positive when the rainfall of the previous week was used. Our rainfall data also were so arranged that the rainfall preceded the unit-leaf-rates by 4 days; thus the rainfall of the week ending on the tenth of the month was correlated with the unit-leaf-rate for the week ending on the 14th. The coefficient obtained was larger than that observed with maize. The direct influence of the water supply upon the rate of photosynthesis has been observed by Dastur (9), and the influence upon the opening and closing of the stomatal pores of the leaf exercised by water relationships is also relevant. Both with maize and barley a positive coefficient was obtained with the solar radiation. Our data did not provide such a result, the somewhat crude method employed of using the length of the sunny period (standard sunshine recorder) regardless of the intensity of the light being no doubt partly responsible.

(b) *Other indices of growth and the climatic factors.*

Before attempting the correlation of other indices with the climatic factors it is necessary briefly to outline the various assumptions involved in the methods. These have been pointed out by Briggs to whose criticism Gregory has replied. Our methods are similar to those of Gregory and merit criticism as follows:

(i) In calculating the mean curve of either the leaf growth or the

dry weight accumulation it is somewhat unlikely that by using four series of data a close approximation to the "normal" can be obtained.

(ii) By this method not only do we assume that the direct effects of the external factors operating at any given time "average out" and allow us to approach the "normal," but further, that the "after effect" can be dealt with similarly. That is to say, that in our observed data we have representative cases of these phenomena, so that the mean curve is built upon such facts. In this connection it is pertinent to consider the results of Maximov (16) obtained with barley and oats with which he demonstrated the prolonged and remarkable effect of germinating the seed at low temperature; it would seem somewhat unlikely that a reasonable approximation to the "normal" can be obtained without using a sufficiently large number of series to include all conditions of germination. If such are the "after effects" of climatic factors operating at one stage it will readily be admitted that before we can construct formulae to embrace all conditions of weather operating on plants of average "tone" the normal curves should be based on many more observations.

(iii) The first direct correlations assume that the influence of a given factor is the same at different stages of growth. In this matter we refer to the work of Alun Roberts (1) who has shown with oats that a particularly sensitive growth stage does exist as far as the subsequent yield is concerned (time of panicle exsertion).

(1) *Relative growth rate—efficiency index.* When the departures from the "normal" growth rate were correlated with the climatic factors the coefficients were not significant. This result caused several methods of arranging the data of the climatic factors to be tried; despite these, the coefficients remained insignificant. Further, the data of the later period of growth only were employed separately, for in this period the relative growth rate varies with the dry weight almost in a linear relationship. With these values of the relative growth rate the correlation coefficients with the climatic factors of the period were also all of no significance. The graphs made of the climatic factors and the growth rate explain to a limited extent the lack of correlation obtained by the direct method. With the series sown in March it was observed that a rise in the maximum (day) temperature was frequently followed by an increase in the growth rate; also a sharp fall in temperature was often accompanied by a fall in the growth rate; but any subsequent rise in the temperature was not immediately followed by an increase in the growth rate—there was a decided "lag." This could be dealt with mathematically and the check to growth

so evaluated, but when we consider the graphs of the plants sown in April these curves cannot be so dealt with, as although a precisely similar fall and subsequent rise in temperature was observed, frequently no decrease in the growth rate occurred. No further series of sowings were available unfortunately, so that this point could not be elucidated satisfactorily. We may postulate several explanations, but unless we can put them to the practical test little advance can be made. However, it would seem that in this one respect our two series (dates of sowing) of plants were not equally "sensitive," and it is somewhat remarkable that there is agreement between the two series sown in different years on the same date in this particular respect. We can only repeat that our experiments, unlike those of Gregory, failed to show us any relationship between the relative growth rate and the climatic factors when the method of calculating the departure from the "normal" rate was employed; perhaps due *partly* to the lack of sufficient data[1] as well as to the inherent assumptions of the method itself, and the field conditions of our experiments.

(2) *The growth of the leaf surface and climatic conditions.* The deviations from the mean rate of growth of the leaf surface were correlated with the climatic factors in a similar manner to that employed for the dry-weight increase. After taking partial coefficients the following were the important significant results:

"*R*" (*a*) and rainfall ...	. temperature and sunshine	0·525 ...	signif.
"*R*" (*a*) and temperature ...	. rain and sunshine	−0·454 ...	signif.

It is therefore seen that the rate of growth of the leaves is closely correlated with the rainfall of the preceding days, whilst high temperatures are associated with slow development of the leaf area. With barley (13) high night temperatures were associated with slow growth of the leaf surface. The coefficients with the length of the period of sunshine were very small and of no significance.

E. THE GENERAL INTERPRETATION OF THE RESULTS.

Despite a high average weekly rainfall the unit leaf rate is closely correlated with the previous rainfall. Much of the rain drains away very rapidly on the stony soil with little "clay" in its composition. From our results with plants competing with one another it is evident that a high rainfall is necessary for assimilation to reach its maximum. The positive correlation with temperature is not in any way surprising, all the applicable evidence indicating that the rate of photosynthesis is accelerated by an increase in the temperature. The rate of growth of the

[1] There were only fifty readings available for correlation calculations.

leaf area is also correlated with the rainfall; therefore both the rate at which the leaf grows in area and the rate at which it works must often depend upon the supply of water from the roots. Consequently, it is somewhat surprising that the rate at which dry matter accumulated in the tops did not also give a positive significant correlation with the rainfall, but no significant coefficient was observed when the efficiency index was employed. The negative coefficient obtained between the rate of leaf growth and temperature explains to a certain degree the lack of any significant correlation between the rate of dry-weight increase and the temperature, for the unit leaf rate was positively correlated with the day temperature. The influence of the period of sunshine could not be detected by any of the indices employed.

The results taken as a whole have demonstrated that despite the errors inherent in the methods of correlation study that have been here employed, it is possible to obtain information which when tested with data concerning the yield—and that is the test of primary importance from the agronomic point of view—will prove reliable.

Comparing our field method and the utilisation of ordinary meteorological data with the pot cultures of other workers, it is seen that we have not obtained such a complete analysis as that shown by Gregory, for example; but it is claimed that our results do show *the relative importance of the factors to the plant in competition with its neighbours;* the method of pot culture, as adopted by others, at once interferes with the water relationships of the plants. The outstanding drawback to the field method is that it is not suitable for plants having a fibrous branched root system, for it is impossible to recover the roots of the large number of plants that are required. The field method should prove more useful with root crops where the great bulk of the root system can be easily recovered. The method of weighing the "tops" only has obvious disadvantages.

Summary.

Previously reported studies with oats have been continued. The present paper deals with the rate of growth of the plants in the field in relation to the meteorological conditions experienced in mid Wales. The method employed was to lift plants at weekly intervals and to measure the leaf area and obtain the dry weight of the top growth.

1. It has been shown that the Unit Leaf Rate (measured on the "tops" of the plants only) is correlated with the previous rainfall and also with the temperature, during that period of growth in which the plants are forming new leaves and increasing their leaf area.

2. The relative rate at which the leaf area increased is correlated also with the previous rainfall; high rainfall ensures leaf development. The rate at which the area increases was negatively correlated with temperature under the field conditions. That is to say that the plant is a cool-loving cereal.

3. When the relative rate at which the dry weight of the tops increased was employed as the index of growth it was not possible to trace out significant relationships with the meteorological data.

4. A comparison with the correlation studies of other workers is also made. Some of the limitations of the method are pointed out, and brief reference to previous criticisms of similar methods employed by other investigators is made. The method has been shown to provide data of a reliable kind, in the case of field grown plants. Previously no such field studies with our common crops had been made.

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EXTRACTS OF PYRETHRUM: PERMANENCE OF TOXICITY AND STABILITY OF EMULSIONS

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(With 5 Text-figures.)

CONTENTS.

Introduction.

Until quite recent times the flowers of pyrethrum (*Chrysanthemum cinerariaefolium*) were generally used for insecticidal purposes as a finely ground powder. Considering the small amount of the active principles

present in the flowers, it is surprising that this method was as effective as it was generally found to be. That the full activity of the poisons did not come into play can hardly be a matter of doubt, and the attempts in more recent years to prepare and employ extracts for spraying purposes has probably led to a much wider field of usefulness for this valuable insecticide.

Until the structure of the two pyrethrins had been elucidated by Staudinger and Ruzicka (8), who have shown them to be esters and liable to decomposition or chemical change detrimental to the insecticidal action, the introduction of rational principles to the making of such spray fluids was hardly to be expected. Staudinger and Harder (7) indicated that loss of toxicity might occur in the alcoholic-soap extracts (known in France and Switzerland as Savon-pyrèthre) by hydrolysis, as neither the acid nor the alcohol portions of the esters are materially toxic to insects, and in addition by alcohol-radical exchanges particularly in the presence of methyl alcohol. Thus a careful choice of solvents and emulsifier is a matter of importance. More inert solvents than alcohol and less reactive emulsifiers and wetters than soap have therefore been suggested. This paper is devoted to an examination of the problem of preparing mixtures, in which the poisons are stable and from which sufficiently stable emulsions can be simply made. It is obvious, however, that only a limited number of the many possible combinations could be examined and therefore a selected number of extracts were prepared.

There are several considerations to be taken into account when framing experiments for the study of this problem: (1) the poisons should not undergo chemical change within a reasonable time, (2) the extract should readily mix with water to form a suitable fluid, *i.e.* the emulsions should be stable enough to be conveniently applied, (3) the spray should not be injurious to foliage.

(1) The active principles of pyrethrum are esters. *Prima facie* it would, therefore, appear that the use of strong alkalies in the mixtures and spray fluids would have to be limited and the extent to which they could be used with safety determined. In addition, the employment of strong emulsions did not seem desirable, and after some experiments on the emulsification of strong pyrethrum-petroleum extracts we decided to devote attention chiefly to petroleum and alcoholic extracts and to the so-called miscible oil preparations, *i.e.* to clear oils which, while they contain a minimum amount of water and are insoluble therein, nevertheless will, under suitable conditions, mix fairly readily with water as oil-in-water emulsions.

(2) Emulsions or extracts of pyrethrum in water-soluble solvents can, in general, be readily diluted. It was, however, found early in this work that some constituent of pyrethrum flowers had a de-emulsifying effect and that extracts made with light petroleum oils and an emulsifying agent could not be worked into the form of stable emulsions with anything like the same ease as the corresponding inert petroleum oil alone. Some of the conditions involved in emulsifying pyrethrum extracts are the subject of a further communication by one of us (R.P.H.). Pyrethrum emulsions might become relatively unstable during transport, and vibration, variation in temperature, etc., might cause a separation of the dispersed phase or a reversion to a water-in-oil condition. Cogency is thus given to the argument in favour of devoting greater attention to the miscible oils, which, being clear solutions, are generally not sensitive to external conditions except that of temperature which can be readily tested in the laboratory.

(3) The use of certain organic solvents in conjunction with pyrethrum is further justified if it can be shown that they too have an insecticidal value or enhance that of pyrethrum. That this is so, particularly in the case of petroleum solvents, has been suspected for some time, and our data lead to a similar conclusion. On the other hand, considerable care has to be exercised in using petroleum products upon foliage. Light fractions, if used above certain concentrations, may have an immediate serious effect, and heavy fractions a delayed but none the less destructive action. The oil used should be of relatively high purity and the concentration in the spray fluid well within the margin of safety. The following is a summary of certain of the findings of deOng, Knight and Chamberlin (5). (*a*) Non-viscous oils of a low boiling point, such as kerosenes, are safer in use on the tree than those of high-boiling points, but are unsatisfactory as scalecides because of relatively low toxicity combined with high volatility. (*b*) Highly refined, white lubricating oils are probably the most advisable for use on citrus trees, especially at summer temperatures. Oils of low viscosity are apparently safer to use on trees than those of high viscosity. (*c*) Severe injury to the citrus tree from the use of lubricating oil is associated with the presence of a high percentage of unsaturated hydrocarbons. (*d*) Gross symptoms of injury to citrus trees may result from the use of unrefined petroleum oils, including defoliation, fruit spotting, dropping and the killing of twigs and branches. (*e*) A quick-breaking emulsion utilises to the maximum degree the insecticidal agent. deOng (6) has also given specifications for petroleum oils to be used on plants. It appears, therefore, that the use of petroleum

in a spray fluid would tend to lower the concentration of the more costly pyrethrum necessary to kill and the effect would be the greater the larger the amount of it that could be employed commensurate with safety to the tree or plant. This amount, however, would be dependent on the petroleum fraction used and its freedom from deleterious substances.

Permanence of the active principles.

As the active principles of pyrethrum are complex esters and are supposed readily to undergo chemical change and hydrolysis, the results of which lessen toxicity, some attention had to be given to the degree of stability of the poisons in certain solvents and in the presence of certain emulsifiers. It was decided to carry out these experiments under two sets of conditions, at the laboratory temperature and at one corresponding approximately to the mean temperature in tropical countries; 28 to 30° C. was chosen as one that could be readily maintained for long periods. Preparations of pyrethrum were, therefore, made and divided into two portions, one of which was allowed to stand in the laboratory, the other placed in a stoppered tube and kept in an incubator adjusted to 28 to 30° C. Attempts were made as far as possible to adjust the concentration of the active principles so that the amounts of solvent and adjuvant would be insufficient when used by themselves to have any serious toxic action on the insects used. In some of the tests, petroleum ether rather than the higher boiling fractions of petroleum was used as giving a greater margin of safety in this regard. Permanent emulsification of petroleum ether extracts of pyrethrum is more difficult than those made with the higher boiling fractions, and this leads to some difficulty in interpreting the results, when no emulsifier has been incorporated.

As our stock of *Aphis rumicis*, the test insect employed, had to be started afresh during the year 1929 from a wild colony, our insects cannot be regarded as standardised to the same extent as in the previous years; and as the experiments have been spread intermittently over 2 years, the results have to be judged with some care in that cross-comparisons from data obtained on different dates should be made with caution, and the effects produced should be compared as far as possible with those obtained on the same day. Owing to variations in insect resistance on different dates, the control tests did not always show absolute blanks, due in certain cases to some slight toxic action of some of the adjuvants; for comparative tests, however, amongst preparations sufficiently alike in composition, this need not lead to erroneous conclusions.

The spray trials were carried out in the way and by the use of the apparatus previously described (11). Observations on the effects of spraying were carried on for 3 days, the results at the end of 3 days being taken except where meteorological conditions were such as to lead to heavy mortalities in the controls, in which case the results at the end of the second day are given (only necessary in one case). The toxic effects were expressed by giving the percentage number of insects falling into the four categories: (1) those not affected, (2) those slightly affected, (3) those moribund, (4) those apparently dead. The examination of the data expressed fully, although preferable as showing grading effects, would make the tables unduly long and cumbersome and therefore in general we have abbreviated them by giving the collected percentages of moribund and apparently dead insects for each concentration.

Pyrethrum flowers. A sample of the flowers, taken from a plot in Harpenden in 1928, was divided and one half ground; both the whole and the ground halves were again divided and each portion placed in a large tube so that it was half-filled by the material, the tubes were then closed by tightly fitting rubber stoppers. One tube of the ground and one of the whole heads were placed in an incubator and kept at 28° C., the other two being allowed to stand at laboratory temperature. In addition a sample of the ground material was spread in a thin layer in a Petri dish and placed in the incubator. Eighteen months later all of the samples were extracted with absolute alcohol, diluted with a 0·5 per cent. solution of saponin and their toxicities to *A. rumicis* determined. The results are presented in Table I. The data given in Table I show that there is little difference after 18 months between the toxicities of the two samples kept in tubes at 28° C. and those allowed to stand at room temperature. In addition the table includes data for a sample of the same pyrethrum tested shortly after harvesting. Too close a comparison with the results obtained for this sample and the above must not be attempted, as it was extracted in a different way and the concentrations tested are slightly different and moreover 2 years have elapsed between the respective tests, but it appears safe to conclude that the results given by it are of the same order and that the stored samples have stood up well to the effect of time and temperature. This, however, only applies to samples stored in closed tubes, for the sample exposed in a thin layer at 28° C. has lost the greater part of its toxicity in 18 months. In addition a commercially prepared pyrethrum dust was found to lose a greater proportion of its insecticidal properties when exposed

in a thin layer at room temperature for a fortnight. As the air in the incubator and the room were comparatively dry, the question arises whether the loss of toxicity could be due to hydrolysis, and it is reasonable to conclude that other factors may be involved.

Table I. *Permanence of toxicity of pyrethrum.*

Series A stood as flowers. Series B stood as 10 % alcoholic extracts of flowers.

Test-subject *Aphis rumicis.*

Description of test	Percent. flowers / ,, pyrethrin I	Percentage moribund and apparently dead insects 3 days after spraying at concentrations of		
	Percent. flowers	0·35	0·2	0·1
	,, pyrethrin I	0·0018	0·001	0·0005
Series A:				
Unground heads at lab. temp. 2 years stoppered tube		100	90	50
Unground heads at 28° C. 18–19 months stoppered tube		100	100	80
Ground heads at lab. temp. 18–19 months stoppered tube		100	100	60
Ground heads at 28° C. 18–19 months stoppered tube		100	80	70
Ground heads at 28° C. 18–19 months in a thin layer		40	20	10
0·5 % saponin solution and alcohol to correspond with		10	0	—
Series B:				
Stood at lab. temp. as 10 % extract in *absolute alcohol* 18–19 months		100	100	80
Stood at 28° C. as 10 % extract in *absolute alcohol* 18–19 months		100	100	75
Stood at lab. temp. as 10 % extract in 95 % alcohol 7½ months		100	100	65
Stood at 28° C. as 10 % extract in 95 % alcohol 7½ months		100	100	60
Stood at lab. temp. as 10 % extract in 95 % alcohol 18–19 months		100	95	72·5
Stood at 28° C. as 10 % extract in 95 % alcohol 18–19 months		100	100	65
0·5 % saponin solution and absolute alcohol to correspond with		10	0	—
0·5 % saponin solution and 95 % alcohol to correspond with		5	0	—
	Percent. flowers	0·44	0·18	0·13
	,, pyrethrin I	0·0022	0·0009	0·0006
*Flowers as above immediately after harvesting		100	80	40

* This test was carried out many months before others in the table, and too close a comparison is not legitimate.

Alcohol extracts of pyrethrum flowers. The extraction of pyrethrum by commercial ethyl alcohol has been practised for some years, in many cases the extract being mixed with soap, *e.g.* in savon-pyrèthre. The latter practice has for some time been regarded as unsatisfactory, owing to the risk of hydrolysis or other chemical change of the esters. (Certain

data on the effect of alkalinity on the stability of these compounds are given later.) It was, however, pointed out by Staudinger and Harder (*loc. cit.*) that some risk was involved in the use of alcohol, particularly in the presence of alkali, as the pyrethrins might undergo an alcohol exchange involving loss of toxicity. We have kept for several years the extracts of pyrethrum flowers, prepared by the use of commercial 95 per cent. alcohol which in field practice retained their toxic action down to comparatively low concentrations (0·5 per cent. in terms of flowers). In laboratory trials samples of the same flowers, the toxicity data of which are given in Table I, were extracted by both absolute and commercial 95 per cent. alcohol. The clear solutions, in both cases, were divided and one-half of each kept at laboratory temperature and at 28° C. in tightly corked tubes. They were tested after 8½ months and again after standing a period of 18 months. The data are given in Table I.

It can be concluded from these data, that in temperate climates, alcoholic extracts of pyrethrum can be prepared with some assurance as to the relative permanence of the toxic effect, and that there is surprisingly little loss of toxicity over long periods at temperatures as high as 28° C. Our results, therefore, indicate loss of toxicity to be inconsiderable, provided the extracts be used in a reasonable time after preparation. Mixing with soap solutions should not, however, take place until immediately before spraying is to be carried out. Alcohol extracts have certain advantages in that they are easy to handle and mix with water of all degrees of hardness without difficulty to give an emulsion of great stability.

Petroleum preparations. Pyrethrum is often extracted by derivatives of petroleum (*e.g.* petroleum ether) and afterwards made up with kerosene or lighter petroleum fractions. For horticultural work, it may become necessary to prepare oil-in-water emulsions of the petroleum extract at high dilutions with water of different degrees and types of hardness. The petroleum extract can be emulsified to some degree of permanence by vigorous stirring with soap solutions in soft water. As such water is not always available in large quantity and as under certain circumstances the reverted or water-in-oil type of emulsion might inadvertently be made, the addition of certain emulsifiers to the oil is recommended for use—yielding so-called miscible oils.

Miscible oils can be prepared by incorporating with the petroleum extract certain sulphonated oils together with alkali. The degree of the permanence of the active principles in the presence of two sulphonated oils was therefore determined. As the pyrethrin esters seemed more

likely to undergo chemical change, especially hydrolysis, in the presence of alkalies of a high degree of dissociation, ammonia in varying proportions was mixed with one of the sulphonated oils and the tests carried out at laboratory temperatures and at 28 to 30° C.

One of us (R. P. H.) has investigated the effect upon interfacial tension of using buffers of different pH with petroleum extracts of pyrethrum(4). He was able to determine the pH at which the stream of extract from a dropping pipette failed to form discrete bubbles and noted that some degree of alkalinity in the water, used for dilution of certain of the miscible oil preparations, rendered emulsification much more easy and rapid. A certain number of tests were, therefore, carried out to ascertain how long the pyrethrins would remain active when dispersed in media of different pH values (p. 224).

Petroleum-ether extracts. Tests were carried out on a 10 per cent. petroleum ether extract of pyrethrum, one portion of which had stood at laboratory temperature and the other at 28° C. for 7½ months and again for a full period of 18 months. The toxicity trials presented very considerable difficulties owing to the separation of the ether from the dilutions with solutions of 0·5 per cent. saponin. There was, however, very little difference in the insecticidal values to be noted between the two samples after 7½ months and again after 18 months—but both samples were less effective at the end of 18 months than they were after 7½ months. It is impossible to state, without further investigation, whether the loss was real or due to a greater insect resistance on the later date, or whether owing to rapid separation the respective concentrations varied on the two dates. It was noted that the sample warmed to 28° C., lost almost the whole of the yellow colour initially characteristic of the extract.

Water-miscible oils containing Agral W.B. A series of trials were carried out with an emulsifier known to commerce as Agral W.B. Certain tests were also made with ammonia oleate for purposes of comparison. The former we found could be used with calcium-hard water, whereas ammonium oleate would necessitate the use of soft waters.

A 20 per cent.[1] extract of pyrethrum (in terms of the flower heads) in technical petroleum ether was mixed with a 25 per cent. solution of Agral W.B., in such proportions as to give a 10 per cent.[1] content of Agral W.B. This mixture was allowed to stand for a period of over

[1] The term per cent. throughout this paper usually has the meaning of gm. per 100 c.c.

6 months both at laboratory temperature and at 28–30° C. The samples were then diluted with 0·5 per cent. saponin in water and tested against a freshly prepared mixture. The results are set out in Table II. In addition, this table (series B) contains data for a mixture of a 20 per cent. solution of Agral W.B. to which had been added 0·2 cc. of 0·902 ammonia to remove a little cloudiness.

Table II. *Permanence of toxicity of petroleum-ether extracts of pyrethrum in the presence of Agral W.B.*

Concentrations expressed in terms of flowers. Test subject *Aphis rumicis*.

Preparation and time of standing	Percentage moribund and apparently dead insects 3 days after spraying at concentrations of 0·35 %	0·2 %	0·1 %
Series A:			
Mixture containing 12 % pyrethrum flowers and 10 % Agral W.B. Stood at lab. temp. 6 months 21 days	100	100	50
Mixture as above. Stood at 28–30° C. 6 months 21days	100	100	70
Mixture as above. Freshly prepared	100	100	30
Control Agral W.B. and solvents to correspond with	10	0	0
20 % pyrethrum extract in petroleum ether (no adjuvants)	100	80	65
Series B:			
Mixture equivalent to 16 % pyrethrum flowers and 20 % Agral W.B. with 0·2 c.c./100 c.c. of ammonia (0·9). Stood at lab. temp. 7 months 3 days	100	50	10
Mixture as above. Stood at 28–30° C. 7 months 3 days	100	70	10
Mixture as above. Freshly prepared	90	80	30
Control Agral W.B. and solvents to correspond with	40	30	20

Series A on cooling to 0–3° C. gave a faint precipitate. Series B were clear.

* Cross references between series A and B should not be made—the insects used for series A were less resistant than those used in series B.

It should be pointed out that comparisons between series A and B cannot be made with advantage, as not only were the mixtures tested on different days but were made up in different ways on different dates. We can deduce from series A of this table that the presence of 10 per cent. of Agral W.B. has little or no harmful effect upon the stability of the active principles of pyrethrum over a period of several months either at laboratory temperature or at 28 to 30° C.

In series B the Agral W.B. at the higher concentration seems to have had a killing effect, due probably to the clogging of the respiratory system; in any case such a result with too high a concentration of a viscous oil is not unlikely to occur. This does not invalidate the deduction from this series that the addition of 20 per cent. Agral W.B. did

not materially lower the toxicity of the samples that had stood over 7 months either at laboratory temperature or at 28 to 30° C.

For the preliminary tests with Agral W.B. and oleic acid treated with ammonia, Agral W.B. and oleic acid were dissolved in technical petroleum-ether and dry ammonia gas passed until supersaturated. In the case of oleic acid the solution became gelatinous and turbid, and in order to clear, an amount of 95 per cent. alcohol was added. Stock solutions equivalent to approximately 25 per cent. of the original Agral W.B. and oleic acid were made up and 20 c.c. of this added to 30 c.c. of an extract of pyrethrum flowers in technical petroleum-ether, making a mixture equivalent to 12 per cent. pyrethrum in terms of flowers, 10 per cent. of Agral W.B. and of oleic acid. Each mixture was divided into two parts, one being allowed to stand at laboratory temperature and the other at 28 to 30° C. The results obtained with dilutions of these mixtures are given in Table III. Owing to very warm weather prevailing at the time of the tests they could not be carried beyond 2 days without risk of exaggerating the toxic effects, and even at the end of 2 days the lowest concentration in the control test with Agral W.B. shows an apparent toxicity. A sufficient number of insects of good quality were not available to test out extracts made up just prior to the tests. Reference to the table shows that the mixture containing Agral W.B. kept at 28 to 30° C. is not completely toxic at the highest concentration used, in contrast with the sample kept at laboratory temperature. It may be deduced therefore that under tropical temperatures this mixture could not be expected to retain its insecticidal properties for long periods. The treatment is, however, very drastic and the toxicities for both samples proved higher than expected.

Ammonium oleate is itself toxic to *Aphis rumicis*, and the results in the table are therefore difficult to interpret. We consider that the mixtures prepared from dry ammonium oleate are not likely to remain unaltered in toxic properties either in temperate or tropical climates. The sample made from oleic acid exactly neutralised by aqueous ammonia and kept at 28 to 30° C. has suffered hardly a significant loss of toxicity as compared with the samples standing at laboratory temperature. It is difficult to say to what extent the toxicity has been enhanced by the presence of ammonium oleate, or how rapid a loss of toxicity of the pyrethrins would result from its use, but seeing that rather careful adjustment of alcohol to petroleum solvent has to be made to prevent turbidity, particularly in the cold, this emulsifier would have to be used with caution.

Table III. *Permanence of toxicity of pyrethrum extract in presence of Agral W.B. (ammoniated) and ammonium oleate.*

Concentration expressed in terms of flowers. Test subject *Aphis rumicis.*

Preparation and time of standing	Percentage moribund and apparently dead insects 2 days after spraying at concentrations of			Effect of cooling to 0–3° C. for 24 hours
	0·35 %	0·2 %	0·1 %	
Extract equivalent to 12 % pyrethrum flowers and 10 % Agral W.B. saturated with dry ammonia gas. Stood at lab. temp. 6 months 3 days	100	40	0	Clear
Extract as above. Stood at 28–30° C. 6 months 3 days	70	40	20	
Agral W.B. and solvents to correspond with	0	0	20	
Extract equivalent to 12 % pyrethrum flowers and 10 % oleic acid saturated with dry ammonia gas. Stood at lab. temp. 6 months 3 days	90	40	40	Separated into two layers
Extract equivalent to 12 % pyrethrum flowers and 10 % oleic acid saturated with dry ammonia gas. Stood at 28–30° C. 6 months 3 days	90	30	10	
Oleic acid saturated with dry NH_3 and solvents to correspond with	20	10	10	
Extract equivalent to 12 % pyrethrum flowers and 10 % oleic acid neutralised with ammonia S.G. 0.9. Stood at lab. temp. 6 months 3 days	100	80	30	Trace of precipitate
Extract equivalent to 12 % pyrethrum flowers and 10 % oleic acid neutralised with ammonia S.G. 0.9. Stood at 28–30° C. 6 months 3 days	90	70	30	
Control. Saponin control 0·5 %. No effect	—	—	—	

Control. Petroleum-ether (comm.) 4 c.c./100 c.c. + 0·5 % saponin solution—20 % dead
" " 2 c.c./100 c.c. " " —no effect
" " 1 c.c./100 c.c. " " —10 % dead

A set of experiments was started to ascertain if there was a correlation between any loss of toxicity and the proportion of ammonia to Agral W.B. used. To weighed amounts of Agral W.B. in petroleum-ether were added increasing volumes of strong ammonia solution (S.G. 0·902) to give ratios of $\frac{0\cdot902 \text{ ammonia (c.c.)}}{\text{Agral W.B. (gm.)}}$ of 0·01, 0·02, 0·05, 0·1, and 0·2; to these solutions were added a 20 per cent. pyrethrum extract in petroleum-ether so as to give 10 gm. of Agral W.B. per 100 c.c. The mixture containing the lowest concentration of ammonia was slightly cloudy immediately after mixing but cleared on standing. The remainder were clear, but the sample containing the highest proportion of ammonia was somewhat viscous. Each mixture was divided, one portion being

kept at laboratory temperature, the other at 28 to 30° C. in an incubator. Their toxicities were determined $5\frac{1}{2}$ months later, and in one case (0·1 ratio) after 18 months. It would be impossible to insert the full tables of data, and therefore summaries are given in Tables IV and V.

Table IV. *Permanence of toxicity of petroleum extracts of pyrethrum containing* 10 *per cent. Agral W.B. and varying proportions of ammonia* (S.G. 0·9).

Concentrations expressed in terms of flowers. Test subject *Aphis rumicis*.

Ratio $\frac{0{\cdot}902 \text{ ammonia c.c.}}{\text{Agral W.B. gm.}}$	Percentage moribund and apparently dead insects 3 days after spraying at concentrations of: 0·35 %	0·2 %	0·1 %	Effect of cooling to 0–3° C. for 24 hours
0·01 stood at lab. temp. 5 months 19 days	90	70	10	Clear
0·01 ,, 28–30° C. ,, ,,	100	100	40*	
0·02 stood at lab. temp. ,, ,,	100	80	10	Clear
0·02 ,, 28–30° C. ,, ,,	100	90	20*	
0·05 stood at lab. temp. ,, ,,	90	70	40	Practically clear
0·05 ,, 28–30° C. ,, ,,	90	70	—	(Trace ppt.)
0·1 stood at lab. temp. ,, ,,	100	70	40	Practically clear
0·1 ,, 28–30° C. ,, ,,	100	100†	—	(Trace ppt.)
0·2 stood at lab. temp. ,, ,,	100	100	—	Clear
0·2 ,, 28–30° C. ,, ,,	100	100	20	
20 % pyrethrum extract in petroleum ether, no Agral W.B. Stood at lab. temp. 5 months 27 days	60	20	—	
0·5 % saponin solution 10 % M. and D.; 0·5 % saponin solution. No M. and D.	—	—	—	
Petroleum ether 2 c.c. in 100 c.c. 0·5 % saponin solution and petroleum ether 1 c.c. in 100 c.c. 0·5 % saponin solution gave no moribund and dead	—	—	—	
Duplicate test after standing 18 *months:*				
0·1 stood at lab. temp.	95	100	50	
0·1 stood at 28–30° C.	100	95	30	

* A certain amount of volatilisation of the petroleum-ether had taken place at 28–30° C. and although this was made up by the addition of more petroleum-ether, there would be a slightly higher proportion of the less volatile petroleum derivatives in these samples. It is reasonable to suppose that this would add slightly to the toxicity.

† The whole of these were moribund and not dead. If marks had been awarded this value would have been approximately the same as the corresponding sample kept at laboratory temperature.

In Table IV the sum of the percentages of moribund and dead insects for the concentrations tested is given for each mixture. This does not bring out the gradations in the toxic effects, but it does make possible a simple and fairly accurate comparison. Inspection of the table leads to the deduction that samples heated to 28 to 30° C. have stood as well

as the samples left at laboratory temperature. Indeed, in one or two cases the former appear more toxic. The differences are probably not outside experimental error, but since at 28° C. the light petroleum evaporated to some extent and penetrated quite close-fitting stoppers (the level had to be made up again to the mark) there would be left in the warmed samples a rather larger proportion of the high-boiling fractions of petroleum, which are suspected of being the more toxic than the lower-boiling fractions. The sample, in which the ratio ammonia/Agral W.B. was 0·1, was again tested after standing for a further 12 months (*i.e.* 18 in all). There seems to have been little or no change in the toxicity of the mixture.

Table V. *Permanence of toxicity of pyrethrum extracts with 10 per cent. Agral W.B. (ammoniated).*

(Summary)

N. = not affected; S. = slightly affected; M. = moribund; D. = apparently dead.

Results 3 days after spraying.

Preparation and time of standing	Concentration in terms of flowers %	N. %	S. %	M. %	D. %	M. and D. %	Effect of cooling to 0–3° C. 24 hours
Series A:*							
Extracts in Table III containing 10 % Agral W.B. and varying amounts of ammonia. Stood at lab. temp. 5 months 19 days (mean values)	0·35	—	4	48	48	96	All practically clear
	0·2	16	6	42	36	78	
	0·1	68	10	22	—	22	
Extracts as above but stood at 28–30° C. (mean values)	0·35	2	—	19	79	98	
	0·2	2	6	64	28	92	
	0·1	76	8	14	2	16	
0·5 % saponin solution 2 tests (mean results)	—	95	—	—	—	—	
Petroleum-ether + 0·5 % saponin solution, 3 tests	—	100	—	—	—	—	
Series B:							
Extract equivalent to 12 % pyrethrum flowers and 10 % Agral W.B. (ammoniated). Ratio $\frac{\text{ammonia (0·9)}}{\text{Agral W.B.}} = 0·2$. Stood at lab. temp. 6 months 13 days	0·35	—	—	10	90	100	Clear
	0·2	–	—	40	60	100	
	0·1	10	10	60	20	80	
Extract as above freshly prepared. Stood 24 hours	0·35	—	—	20	80	100	
	0·2	—	30	30	40	70	
	0·1	30	10	30	30	60	
0·5 % saponin solution, 2 tests (mean)	—	100	—	—	—	—	

* Cross-comparison between series A and B cannot be made. It was shown by tests with a pyrethrum extract free of adjuvants that the insects used in series B were slightly less resistant than those used for series A.

In Table V the results given in Table IV for the samples kept at laboratory temperature and those kept at 28 to 30° C. are collected together and averaged. In addition, at a later date, the sample containing the highest ratio of ammonia solution to Agral W.B. kept at laboratory temperature was tested against one freshly prepared. These two series are labelled A and B; they should not be inter-compared, as tests carried out on each day with a 20 per cent. extract of pyrethrum, free of adjuvants, showed that the insects were less resistant in series B than in A. From series A it would appear that the sample kept at 28° C. is not less toxic than the one stored at laboratory temperature. Series B presents a curious problem—the freshly prepared sample is apparently somewhat less toxic than the stored sample. It is highly questionable whether this difference is outside experimental error; it should be pointed out, however, that when the sample of ammoniated Agral W.B. used for the extract which stood for 6 months was prepared, some time elapsed before it was mixed with the pyrethrum extract and the resulting product was viscous; with the sample prepared 24 hours previous to testing, this waiting period was very short and the resulting mixture not so viscous. Complete combination between base and oil may not have taken place and some free ammonia may have had a slightly adverse effect on the toxicity in 24 hours. It would appear advisable to allow the ammonia to stand sufficiently long with the Agral W.B. to ensure complete interaction before mixing with the extract of pyrethrum.

A fair deduction to draw from these data is that Agral W.B. can be mixed with petroleum extracts of pyrethrum with comparative safety, that these mixtures can be stored in temperate climates for some time and for several months under tropical and semi-tropical conditions, without much loss of toxicity. The addition of strong ammonia solution to the Agral W.B. to aid clearing and emulsification, provided that time has been allowed for complete reaction between the two, appears from our data to be comparatively safe, if the proportion of ammonia solution (S.G. about 0·9) to Agral W.B. is not greater than about 1 to 5. These tests only apply to mixtures containing 10 per cent. Agral W.B.

Owing to the low toxicity of petroleum-ether to *A. rumicis*, this solvent was used as the organic solvent in the foregoing experiments. In actual practice this solvent would prove unsuitable, as it is too volatile and inflammable and in addition stable emulsions are not easy to prepare from it. Higher fractions such as refined and semi-refined white spirits, kerosene and refined lubricating oils would prove more

generally useful. It would be necessary, however, to use them at concentrations in the tank at which they would be non-injurious to foliage. We regard a concentration of about 1 per cent. to be in general as high as it is safe to use these oils. Agral W.B. is more readily soluble in the lighter fractions such as refined and semi-refined white spirit (S.G. 0·782 and flash point 94° C.), but the higher fractions would be expected to enhance toxicity to a greater extent.

Table VI. *Permanence of toxicity of pyrethrum extracts in the presence of increasing amounts of ammoniated Agral W.B.*

$$\left(\textit{Ratio } \frac{0{\cdot}9 \textit{ ammonia}}{\textit{Agral W.B.}} = 0{\cdot}1\right).$$

Strong extracts have stood 9 months 1 week except where stated.
Concentrations of extracts sprayed are in terms of pyrethrin I.
Test subject *Aphis rumicis*.

Percentage amount of ammoniated Agral W.B. in pyrethrum extract before dilution	Percentage moribund and apparently dead insects 3 days after spraying at concentrations of		
	0·001 %	0·0005 %	0·00025 %
5 % at lab. temp.	100	90	40
5 % at 28–30° C.	100	80	40
*10 % at lab. temp.	100	100	55
*10 % at 28–30° C.	100	80	60
*10 % *prepared* 1 *month*	100	80	40
20 % at lab. temp.	100	100	35
20 % at 28–30° C.	100	90	50
20 % *freshly prepared*	100	70	20
40 % at lab. temp.	100	100	45
40 % at 28–30° C.	90	70	40
Controls with mixtures as above but without pyrethrum gave blanks			
Duplicates after 8 *months' standing:*	0·002 %	0·001 %	0·0005 %
10 % at lab. temp.	100	100	60
10 % at 28–30° C.	100	100	70
10 % freshly prepared	100	100	80
Controls	10	—	10

* These samples were tested a month previously—no significant differences were detected in their toxicities, the data are given lower in table.

Note. The above mixtures when cooled to 0° C. gave a slight deposit after 2 days. In the case of the mixture containing 40 % ammoniated Agral W.B. there was rather much deposit.

A series of tests was made in which the ammoniated Agral W.B. was used in the successively increasing amounts of 5, 10, 20 and 40 per cent. The ratio of 0·9 ammonia (c.c.) to Agral W.B. (gm.) was in each case 0·1, semi-refined white spirit was used as solvent and the concentration of the pyrethrum extract adjusted to bring the content of

pyrethrin I to 0·5 per cent. Each preparation was divided into two parts, one of which was kept at room temperature and the other allowed to stand at 28° C. for just over 9 months. They were then tested for their toxicity to *Aphis rumicis* and compared with recently prepared samples containing 10 to 20 per cent. of the ammoniated emulsifier. The data are set out in Table VI.

The data demonstrate that the loss of toxicity in these preparations at either temperature has not been any greater than the loss in the same period in the concentrated extract of pyrethrum in petroleum-ether (4·5 per cent. pyrethrin I) from which they were made. There may have been some loss of toxicity of questionable significance at 28° C. in the case of the sample containing 40 per cent. of the emulsifier, an amount hardly likely to be used in practice.

A series of tests with lubricating oil and pyrethrum were incorporated to ascertain the stability of the poisons and the degree to which the toxicity was increased by the heavy oil. We do not suggest that the mixture experimented with is the most suitable that could be made; it could, however, be emulsified with rather vigorous stirring with both soap and saponin solutions. In some field trials against a species of capsid bug (*Lygus pabulinus* Linn.) on red currants, the lubricating oil-pyrethrum mixture when emulsified with soap gave good results, practically complete control being established at low concentrations. The following was the method of preparation. A highly concentrated petroleum-ether extract of pyrethrum flowers was prepared for us and further concentrated to a rather viscous fluid. The volatile acid after saponification was determined in the way outlined by us and the amount found calculated to pyrethrin I (10). It gave a figure of 4·5 gm. per 100 c.c. The use of so concentrated an extract allowed highly diluted emulsions to be made, and thus the amount of lubricating oil used for spraying could be cut down to concentrations below that likely to kill the insects when used by itself in control tests, or do damage to foliage. 10 gm. of Agral W.B. to which had been added 0·5 c.c. of strong ammonia (S.G. 0·9) were incorporated with about 85 c.c. of the lubricating oil and 11·1 c.c. of the strong pyrethrum extract. 4 c.c. of oleic acid were added to correct a slight turbidity noticed on cooling to 0° C. With different proportions of ammonia or other heavy oils the amount of oleic acid added would need modification, but it should be cut down to a minimum required to clear. The lubricating oil used was known as refined 50 grade. The mixture was divided into two portions, one of which was kept at laboratory temperature and the other at 28 to 30° C. for 5 months. Tests

were carried out in the usual way by preparing dilutions with 0·5 per cent. saponin—the concentration used being 0·001, 0·005 and 0·00025 gm. per 100 c.c. in terms of pyrethrin I. In addition, tests were made with a sample prepared in the same way the day before spraying and with the highly concentrated stock extract of pyrethrum used in its preparation.

Table VII. *Toxicities to* A. rumicis *of mixtures of pyrethrum extracts and lubricating oil.*

N. = not affected; S. = slightly affected; M. = moribund; D = apparently dead.

Results 3 days after spraying.

Preparation	Concentration pyrethrin I as determined from volatile acid	N. %	S. %	M. %	D. %	M. and D. %
*Pyrethrum-lubricating oil mixture containing 10 % ammoniated Agral W.B. $\frac{\text{ammonia}}{\text{Agral W.B.}}$ = 0·05. Stood at lab. temp. 5 months	0·001	—	—	10	90	100
	0·0005	20	—	10	70	80
	0·0025	60	—	20	20	20
Ditto. Stood at 28–30° C. 5 months	0·001	—	—	10	90	100
	0·0005	30	10	20	40	60
	0·0025	70	10	—	20	20
Ditto. Freshly prepared	0·001	—	—	20	80	100
	0·0005	10	20	20	50	70
	0·0025	40	20	10	30	40
Agral W.B. and all solvents as used in highest concentration	—	100	—	—	—	—
0·5 % solution of saponin	—	100	—	—	—	—
Strong extract of pyrethrum in low boiling petroleum-ether used in above mixtures (no lubricating oil or Agral W.B. present)	0·002	—	—	20	80	100
	0·001	—	40	40	20	60
	0·0005	70	10	—	20	20

* On cooling to 0–3° C. for 24 hours this mixture remained clear.

The control tests with 0·5 per cent. saponin solution and the lubricating oil and ammoniated Agral W.B. gave completely negative results. It will, however, be observed that the pyrethrum extracts containing lubricating oil and Agral W.B. are significantly more toxic than the petroleum-ether extract from which they were prepared. It is thus clear that these higher fractions of petroleum enhance the toxicity of pyrethrum, even when the petroleum is present in sub-lethal concentrations. There is little difference in toxicity between the three pyrethrum-lubricating-oil tests and any discrepancies observed cannot be regarded

as outside experimental error. Pyrethrum would therefore appear to be relatively stable in such extracts as the above, particularly in temperate climates.

Turkey-red oil series. Sulphonated castor oil has been known for some considerable time as an emulsifier, and neutralised material has been suggested by Harder (2) for use with benzene, petroleum-ether, and trichlorethylene extracts of pyrethrum. Turkey-red oil is more soluble in water than neutral Agral W.B., but it is less soluble in organic solvents, therefore miscible oils prepared with it require most careful balancing of solvent to oil if the mixture is to remain clear. Mixtures prepared from turkey-red oil, however, pass into water much more readily than those in which Agral W.B. is used, and have the advantage, as is shown below, of mixing well with magnesium-hard water. Separation may in some cases be comparatively rapid, but gentle stirring of the spray mixture in the tank will keep the emulsion sufficiently uniform for spraying purposes. Although mixtures prepared from turkey-red oil can be used without a wetter they would appear to require its addition in the spray tank in order to secure maximum efficiency. Commercial turkey-red oil is usually alkaline in reaction; it is therefore advisable to neutralise before using it in the presence of the pyrethrins. Harder (2) suggests for this purpose the addition of a little 50 per cent. acetic acid, but it should be possible for neutrality to be secured by the manufacturer by a suitable washing process. One sample used by us was neutral but contained much wash liquor which gradually settled. The sample was therefore allowed to stand for some time and the clear oil carefully decanted off. Another sample was distinctly alkaline in reaction and before use was mixed with an equal volume of refined oleic acid—the product was then neutral to litmus. The addition of oleic acid enables a larger amount of petroleum spirit to be incorporated, but it introduces the disadvantage that the mixture now no longer disperses freely in water, a difficulty, however, that can be overcome by rendering the latter slightly alkaline by the addition of a little sodium carbonate. The turkey-red oil—oleic acid mixture, moreover cannot be used with magnesium-hard water, clotting and reversal of phase taking place. The petroleum fraction used was semi-refined white spirit with the following characteristics:

Specific gravity at 60° F.	0·782
Flash point (Abel)	94° F.
About 78 per cent. distilled below ...	175° C.

It contained a certain portion of aromatic hydrocarbons. Three preparations of the following composition were tested:

A.[1]	Concentrated pyrethum extract in petroleum-ether	11·1 c.c.
	Semi-refined white spirit	18·9 c.c.
	Neutral turkey-red oil to bring to a volume of ...	102 c.c.

B.[1] As A, but 18·9 c.c. of a mixture of tetrachlorethane 40 c.c. and semi-refined white spirit 60 c.c. were used. (This gives a higher specific gravity and so prevents the oil rising quickly to the surface.)

C.	Concentrated pyrethrum extract in petroleum-ether	11·1 c.c.
	Alkaline turkey-red oil	16·6 c.c.
	Oleic acid	16·6 c.c.
	Semi-refined white spirit to a volume of	100 c.c.

These mixtures were divided into two portions, one being kept at laboratory temperature, the other at 28 to 30° C. After periods of time given in Table VIII A and VIII B each was diluted with a solution of 0·5 per cent. saponin in water and tested against a recently prepared sample. The data are given in Tables VIII A and VIII B.

Inspection of Table VIII A and VIII B indicates that the control tests with turkey-red oil and the solvents alone have lethal properties, particularly where tetrachlorethane has been used. Some of this effect is probably mechanical, due to the turkey-red oil, but in addition some toxicity would be expected from tetrachlorethane. Although on this day no trials were possible with the concentrated pyrethrum extract used in the preparation of these mixtures, it is safe to say the turkey-red oil and solvents have enhanced the toxicity of pyrethrum, and although the samples A and B which have stood at the temperature of the laboratory show no loss of toxic properties as compared with the freshly prepared sample, both the samples kept at 28° C. are probably significantly lower in toxicity. Preparations of the type A and B would apparently retain their toxicity in temperate climates over a sufficiently long period.

In addition to the tests after a period of 5 months a further series was carried out with mixture A after 15 months, the results of which are set out in Table VIII B. These tend to confirm the deduction drawn from the data in Table VIII A. It is, however, surprising that after so

[1] A and B separated out a thin layer on surface on standing. The amount of petroleum present has not been adjusted sufficiently exactly.

long a period alkali-free turkey-red oil has so little detrimental action upon the toxicity of the pyrethrins.

Table VIII A. *Permanence of toxicity of pyrethrum turkey-red oil preparations.*

N. = not affected; S. = slightly affected; M. = moribund; D. = apparently dead.

Results 3 days after spraying.

Preparation		Concentration pyrethrin I as determined from volatile acid	N. %	S. %	M. %	D. %	M. and D. %
Mixture A (see p. 221). Stood at lab. temp. 4 months 29 days	(1)	0·002	—	—	—	100	100
	(2)	0·001	—	—	—	100	100
	(3)	0·0005	—	10	20	70	90
Mixture A. Stood at 28–30° C. 4 months 29 days		0·002	—	—	20	80	100
		0·001	—	—	30	70	100
		0·0005	60	10	—	30	30
Mixture A (freshly prepared)		0·002	—	—	—	100	100
		0·001	—	—	10	90	100
		0·0005	—	15	25	60	85
Control with turkey-red oil and all solvents used in	(1)	—	90	—	—	10	10
	(2)	—	80	—	10	10	20
	(3)	—	100	—	—	—	—
Mixture B (see p. 221). Stood at lab. temp. 4 months 29 days	(4)	0·002	—	—	—	100	100
	(5)	0·001	—	—	—	100	100
	(6)	0·0005	10	10	40	40	80
Mixture B. Stood at 28–30° C. 4 months 29 days		0·002	—	—	—	100	100
		0·001	—	10	50	40	90
		0·0005	30	30	—	40	40
Control with turkey-red oil and all solvents as in	(4)	—	40	10	—	—	—
	(5)	—	100	—	—	—	—
	(6)	—	80	—	10*	10*	20
0·5 % solution of saponin		—	100	—	—	—	—

* These insects were of poor quality.

Both mixtures A and B became slightly turbid on cooling to 0–3° C. for 24 hours. On standing at low temperature they separated out a thin layer on surface; adjustment of components had not been exact enough.

Table VIII B, contains data concerning the permanence of toxicity of mixture C, in which the alkalinity of a commercial sample of turkey-red oil was more than neutralised by the addition of oleic acid. Oleic acid and its soap are known to have a lethal effect upon *Aphis rumicis*; this, however, was not pronounced in the controls at the concentrations tested, although it would appear evident that the toxicity of the pyrethrins has been enhanced by its presence. The data show that there has been little or no loss of toxicity of the mixture after standing 6–7 months either at the laboratory temperature or at 28 to 30° C.

Table VIII B. *Permanence of toxicity of pyrethrum turkey-red oil preparations.*

N. = not affected; S. = slightly affected; M. = moribund; D. = apparently dead.

Results 3 days after spraying.

Preparation		Concentration pyrethrin I %	N. %	S. %	M. %	D. %	M. and D. %
Series A:							
Mixture A (see p. 221). Stood at lab. temp. 15 months	(1)	0·002	—	—	—	100	100
	(2)	0·001	10	—	10	80	90
	(3)	0·0005	60	10	10	20	30
Mixture A. Stood at 28–30° C. 15 months		0·002	—	—	10	90	100
		0·001	20	—	—	80	80
		0·0005	90	10	—	—	—
Mixture A (freshly prepared)		0·002	—	—	—	100	100
		0·001	—	—	30	70	100
		0·0005	20	—	40	40	80
Control solvents etc. as	(1)	—	90	—	10	—	10
	(2)	—	90	10	—	—	—
	(3)	—	90	—	—	10	10
Series B:							
*Mixture C (see p. 221). Stood at lab. temp. 6–7 months	(3)	0·001	—	—	—	100	100
	(4)	0·0005	10	5	20	65	85
	(5)	0·00025	40	5	10	45	75
Mixture C. Stood at 28° C. 6–7 months		0·001	—	—	—	100	100
		0·0005	10	10	15	65	80
		0·00025	25	—	15	60	75
Mixture C (recently prepared)		0·001	—	—	10	90	100
		0·0005	—	—	5	95	100
		0·00025	40	—	10	50	60
Control solvents etc. as	(3)	—	95	—	—	5	5
	(4)	—	85	—	—	15	15
	(5)	—	85	—	5	10	15

* Mixture C shows only a slight deposit on cooling to 1° C.

Mixture C goes into water far less readily than either A or B, and, as will be shown later, requires for stable emulsification an addition of alkali to the dispersion medium. It can then be used with calcium-hard waters; magnesium-hard waters, however, cause a reversal of phase to the water-in-oil state, in marked contrast to their effect on the mixtures A and B.

Permanence of poisons in dilute emulsions. It will be shown later that not only does the presence of a weakly dissociated alkali in the miscible oils prepared from Agral W.B. aid the readiness of emulsification and stability of the emulsion formed, but for both of these purposes a certain degree of alkalinity in the water of the spraying tank is desirable. It was, therefore, considered important to ascertain how long poisons of pyrethrum would remain active when dispersed in water of

different degrees of alkalinity. Accordingly, an extract of pyrethrum was added to solutions of 0·5 per cent. saponin buffered at *p*H 7, 9 and 11. The concentration in terms of pyrethrin I, as calculated from the volatile acid, was 0·002 gm. per 100 c.c. After standing, this emulsion was tested, as were also dilutions of 0·001 and 0·0005 gm. per 100 c.c. Tests were made with samples which were prepared just before spraying and with others that had remained in contact with the buffered dispersions medium for 27 hours and 25 days. One series of tests was carried out with a sample which had stood for 25 days in the presence of *N*/10 soda. Unfortunately, it was found that the buffer used had a considerable but delayed toxic action, and therefore the numerical data were of little value. It was, however, noticed that all the buffered mixtures ranging from *p*H 7 to *p*H 11 showed the almost instantaneous narcosis characteristic of the physiological action of pyrethrum. The preparations made with *N*/10 soda showed a loss of toxicity after 25 days.

Table IX. *Stability of the active principles of pyrethrum when dispersed in alkaline solutions.*

N. = not affected; S. = slightly affected; M. = moribund; D. = apparently dead.

Results 2 and 3 days after spraying.

Condition of experiment	Concentration pyrethrin I as determined from volatile acid	After 2 days					After 3 days				
		N. %	S. %	M. %	D. %	M. and D. %	N. %	S. %	M. %	D. %	M. and D. %
N/10 soda unbuffered. Stood at lab. temp. 5 days	(1) 0·002	—	—	—	100	100	—	—	—	100	100
	(2) 0·001	10	20	40	30	70	50	—	10	40	50
	(3) 0·0005	90	—	—	10	10	80	—	—	20	20
N/10 soda unbuffered. Stood at lab. temp. about 24 hours	0·002	—	—	—	100	100	—	—	10	90	100
	0·001	—	20	30	50	80	30	20	—	50	50
	0·0005	50	20	20	10	30	50	20	—	30	30
N/10 soda unbuffered. Prepared just before spraying	0·002	—	—	—	100	100	—	—	—	100	100
	0·001	—	10	10	80	90	—	10	10	80	90
	0·0005	—	10	40	50	90	50	20	20	10	30
Control as No. 1 but no pyrethrum present	—	70	—	20	10	30	70	—	10	20	30
Control as No. 2 but no pyrethrum present	—	100	—	—	—	—	80	—	—	20	20
Control as No. 3 but no pyrethrum present	—	100	—	—	—	—	70	—	—	30	30
*p*H 11 buffered by glycine. Stood at lab. temp. 5 days	0·002	—	—	—	100	100	—	—	—	100	100
	0·001	—	—	50	50	100	—	10	20	70	90
	0·0005	20	—	30	50	80	30	—	40	30	70*
*p*H 11 buffered by glycine. Stood at lab. temp. 24 hours	0·002	—	—	—	100	100	—	—	—	100	100
	0·001	—	—	20	80	100	—	10	50	40	90
	0·0005	10	30	30	30	60	50	20	—	30	30
*p*H 11 buffered by glycine. Prepared just before spraying	0·002	—	—	—	100	100	—	—	—	100	100
	0·001	—	—	—	100	100	—	10	10	90	100
	0·0005	—	—	30	70	100	10	10	—	80	80

* This value probably slightly exaggerates toxicity; there were a large number of moribund.

A fresh series of experiments were set up with the dispersion medium at pH 11, Sorensen's glycine caustic soda mixture being used as buffer, and with $N/10$ caustic soda. The insects had to be evaluated on the second day after spraying, as the control tests showed losses after that period (the third day's results are also given in Table IX); also it should be noted that the $N/10$ soda produced a number of deaths, probably due to its irritant action.

The data in Table IX do not allow of strictly quantitative comparisons owing to the poor quality of the control tests. It may be safely concluded, however, that after 5 days and even after 24 hours in the presence of $N/10$ soda there is with pyrethrum some loss of toxicity; the fact that there is so little difference between the results obtained after 24 hours and those after 5 days is probably due to the partial neutralisation of the soda by resin acids present in the extracts. There is some indication also of a slight loss when the alkalinity is reduced to pH 11. It is, however, a matter of surprise that the loss of toxicity is so small at these degrees of alkalinity and as such highly caustic spray fluids as that given in this case by $N/10$ soda are not likely to occur in practice, it would appear that pyrethrum extracts would show a relatively small decline under normal circumstances after remaining in the spray tank for a few days. As, however, loss of toxicity is likely to take place progressively the diluted spray mixture should not be left too long in the tank.

EFFECT OF COOLING ON WATER-MISCIBLE OILS.

It is important that miscible oils should remain comparatively clear on cooling to temperatures of about 0° C. This problem has been studied by Hart (3) who, for a variety of such oils, traces out the factors necessary for clarity to be maintained at low temperatures, and drew for acid sulphonated castor oil mixtures the following conclusions: (1) in the absence of alcohol the addition of alkali at first decreases and then increases the amount of free oleic acid required for a homogenous product; (2) if alcohol is present in sufficient quantity the more alkali present less oleic acid is required to clear the oil; (3) the more neutralised the sulphonated oil the better it functions as emulsifier, the completely neutralised oil being best in this respect. Oleic acid in proper balance often acts as a clearing agent in miscible oils. Hart's paper is of importance to those interested in the preparation of these oils.

The toxicity data presented in Tables VIII A and VIII B in connection with neutral sulphonated castor oil (turkey-red oil) were for

rather extreme conditions, so as to obtain maximum effects on toxicity and as no oleic acid was added only a minimum of light petroleum oil could be used; but it should be pointed out that the conditions under which these oils may be used, for example in calcium and magnesium-hard water, demands that for clearing purposes a minimum amount of oleic acid should be used in order to avoid clotting and possible reversion. Except in one case (mixture C, p. 221) the use of a considerable proportion of free oleic acid has been avoided in our mixtures.

All our miscible oil preparations were subjected to cooling to temperatures of 0°–3° C. in an "Electrolux" refrigerator for 24 hours, the effect of cooling being indicated in the tables. All oils of this type on being subjected to cooling to 0° C. should remain fairly clear, otherwise, there is risk, through the formation of two phases or the separation of some of the constituents, of the effectiveness being lost. A trace of turbidity is hardly likely to be of importance unless it leads to extensive phase separation. In our tests the only sample to show extensive separation was one containing oleic acid saturated with dry ammonia gas, and it is highly probable that a change in proportion of alcohol to petroleum-ether would have acted as a correction. The turkey-red oil samples A and B showed some turbidity, and it is probable that the ideal combination of petroleum and sulphonated oil was not realised and certain of the Agral-ammonia samples deposited a trace of precipitate, but with pyrethrum extracts such precipitates are probably traces of resin acids and they are not likely to be of importance.

Readiness of emulsification of pyrethrum extracts and stability of emulsion.

For the purpose of investigating the emulsibility of pyrethrum extracts and the stability of the resulting emulsions, we have used as far as possible solutions in which the stability of the poisons had been tested as already described.

In a separate publication one of us (R.P.H. (4)) carried out an investigation of the interfacial tension of pyrethrum extracts against aqueous solutions, with particular reference to the concentration of hydrogen and calcium-ion; a matter of importance as hard waters may be used in making up spray fluids. While a low interfacial tension is conducive to spontaneous emulsification it does not necessarily imply that the resulting emulsion is stable, and it therefore appeared necessary to make up emulsions under the conditions suggested by the study of interfacial tensions and to test their actual stability. The oil content of emulsions

left to stand various periods was estimated by a technique which consisted essentially in breaking the emulsion with acid, the oil being separated and its volume measured by the Gerber method for the estimation of fat in milk.

EMULSIFICATION OF PYRETHRUM EXTRACTS IN HARD WATERS.

Harpenden tap-water was used in these experiments. It was found by analysis to contain 27·6° temporary hardness and 30° total hardness only traces of magnesium were present. The temporary hardness was in addition determined in samples taken over several days by the soap method. On only one occasion was it found to fall below 26°. For testing out higher concentrations of calcium and the effects of magnesium salts calcium and magnesium sulphates were added respectively.

Preliminary test-tube experiments. Solutions were made up in tap-water as follows:

1.	0·1	per cent. Na_2CO_3	
2.	0·1	,, ,,	0·5 per cent. Agral I[1]
3.	0·075	,, ,,	,, ,,
4.	0·05	,, ,,	,, ,,
5.	0·025	,, ,,	,, ,,

0·5 c.c. of the various miscible oils dealt with above were added to 10 c.c. of the aqueous solution and, after shaking, the mixture was left to stand. All the mixtures made with the pyrethrum extract solution without Agral W.B. broke in a few minutes; with the pyrethrum extract solution containing 10 gm. per 100 c.c. of Agral W.B. the emulsions broke rapidly except at the highest concentration of sodium carbonate, 0·1 per cent.; this was the concentration at which Hobson (*loc. cit.*) found the interfacial tension became immeasurable. In this case two of the factors for stability are (1) very low interfacial tension, (2) the presence of a stabilising agent. It is possible that the necessity of adding an emulsifying agent might be avoided by the use of a higher concentration of sodium carbonate, but this was not tested, as a minimum of alkalinity was desirable.

To test out the effect of the magnesium and of calcium in larger amounts, solutions of the sulphates were made up in tap-water. A solution containing pyrethrum extract and 10 gm. per 100 c.c. of Agral W.B.

[1] Agral I is a proprietary article which lowers the surface tension of water and so aids wetting.

was used and the stability of the emulsions observed. With only calcium present it was found that stable emulsions could be formed provided sufficient sodium carbonate or sodium phosphate were added; the precipitate did not settle out or in any way interfere with the emulsion. With solutions of magnesium salts containing various amounts of sodium carbonate, phosphate or hydroxide no stable oil-in-water emulsions resulted; either oil or the water-in-oil phase separated. The failure in the case of sodium carbonate was probably due to the relatively high solubility of magnesium carbonate; the ratio $[Mg^{++}]/[OH']$ is probably important, as the ratio $[Ca^{++}]/[OH']$ was found by Hobson (*loc. cit.*) to influence the interfacial tension. When sodium phosphate or hydroxide or ammonia was added, the magnesium was precipitated and quickly formed a curd which rose to the surface and broke the emulsion. A few experiments were made using casein and gelatine as emulsifiers where magnesium was present; the results, although better, were not sufficiently encouraging to justify continuation of the experiments.

Quantitative determination of the relative stability of the emulsions.

The preliminary experiments indicated that no grave difficulties arise with water containing calcium only, but that the problem with magnesium present is far more serious. To obtain further and more exact data a quantitative technique was worked out.

Method. The emulsions were prepared and poured into a 400 c.c. pear-shaped separating funnel, identical funnels being used throughout. At intervals 25 c.c. were run out slowly and 20 c.c. taken for determination of the oil content. Although a gradient in the oil concentration with depth probably existed, the small proportion of the total volume taken would minimise any error, which would also fall evenly and not affect the comparative accuracy of the results.

The amount of oil remaining in suspension was determined by de-emulsifying and measuring the volume of the oil. The apparatus and principles of the Gerber method of estimating butter-fat in milk was employed. It may be noted that Griffen and Richardson (1) adapted the Babcock method of determining butter-fat in milk for a similar use with oil-sprays. Our procedure was as follows: 20 c.c. of the emulsion were placed in a Gerber butyrometer tube and 2 c.c. of the acid mixture were added. The acid mixture contained 10 per cent. hydrochloric acid and 10 per cent. sodium chloride and 1 per cent. ferric chloride. The excess of

carbon dioxide was shaken out and the rubber bungs pressed in tightly, by whirling in a centrifuge most of the oil was separated; after standing overnight and centrifuging again, the oil was completely separated, the water layer being crystal clear. Occasionally it was necessary to allow the tubes to stand for a further few hours. The volume of oil was read off in the units on the scale, which correspond to 1 per cent. fat in 11 c.c. of milk and which have been calculated to be approximately 0·125 c.c. in volume. Thus if the density of butter-fat at 70° (at which readings of butter-fat are made) is taken as 0·88, then the volume of 1 unit (v) is as follows:

$$\frac{v \times 0{\cdot}88}{11} = \frac{1}{100},$$

$$v = 0{\cdot}125 \text{ c.c.}$$

This value was confirmed by determination with freshly shaken emulsions of known strength.

In most of the experiments a solution of 5 per cent. pyrethrum extract in semi-refined white spirit was emulsified in Harpenden tap-water containing 0·1 per cent. sodium carbonate and 0·5 per cent. Agral I, various substances being added to each phase. The concentration of oil at the start was 2 per cent., except in a few cases when it was 1 per cent. This amount is rather higher than would in general be employed for spraying, but allows more accurate reading on the Gerber tubes. In our experiments there is probably a certain experimental error introduced by the absorption of water by the oil film, but on the whole the results are comparable with each other.

Results. The results are contained in Table X. The main points examined were the influence of the method of making up the emulsion, of the presence of the ammonia and the concentration of Agral W.B. in the pyrethrum solution, and of the presence of varying amounts of calcium and magnesium in the aqueous phase. The results will be discussed in this section under these main heads and in addition a few experiments with turkey-red oil solution will be described. Analyses were carried out with most of the mixtures after intervals of 1, 6 and 24 hours and the general type of curve obtained is shown in Diagram 1, in which the percentage of oil remaining in suspension is plotted against time. An apparent equilibrium is reached in 24 hours, after which the separation of oil becomes immeasurably slow. The rate at which this equilibrium is reached is nearly the same in all cases, and examination of Table X shows that it is immaterial whether comparisons are made on the basis of the results after 1, 6 or 24 hours.

Table X. *Stability of emulsions prepared from water-miscible petroleum spirit extracts of pyrethrum.*

	Oil phase. Pyrethrum solution containing					Percentage of the oil added found in emulsion after			
	Ammonia % (vol.)	Agral W.B. %	Method of preparation	Percentage oil in emulsion	Aqueous phase	¼ hr.	1 hr.	6 hrs.	24 hrs.
Series A:									
1	0	10	A	2	Tap-water containing 30° of	<3	—	—	—
2	0	10	A*	2	hardness as calcium, 0·5 %	20	—	—	—
3	0	10	B	2	Agral I and 0·1 % sodium car-	6	—	—	—
4	0	10	C	2	bonate being added	58	—	—	—
5	0	10	C	1		81	—	—	—
6	0	10	C	1		81	62	45	44
7	0	10	C	2		64	48	34	31
8	(Solvent)	10	C	2		—	63	42	30
9	1	10	A	2		—	89	67	47
10	2	10	A	2		—	72	42	36
11	2	10	C	2		—	48	38	33
12	2	10	B	2		—	44	30	27
13	4	20	B	2		—	80	66	52
14	4	20	A	2		—	85	72	60
15	2	20	A	2		—	89	75	64
16	2	40	A	2		—	76	66	60
17	5	40	A	2		—	95	93	91
18	3·5	25	A	2		—	83	68	61
19	3·5	30	A	2		—	92	81	77
20	2	16	A	2		—	81	—	—
21	5	40	A	2	Ditto + 10° Mg	—	80	—	—
22	5	40	A	2	,, + 20° ,,	—	72	—	—
23	5	40	A	2	,, + 50° ,,	—	42	—	—
24	5	40	A	2	,, +100° ,,	—	20	—	—
25	5	40	A	2	100° Mg, 0·5 % Agral I: 0·1 % NaOH	—	59	—	—
26	5	40	A	2	0·3 % Na_2CO_3	—	30	—	—
27	2	20	A	2	0·1 % Na_2CO_3	—	3	—	—
28	2	20	A	2	0·2 % Na_2CO_3	—	5	—	—
29	2	20	A	2	0·1 % Na_2CO_3, 0·1 % amm. oxalate	—	8	—	—
30	5	40	A	2	100° Ca, 0·5 % Agral I: 0·1 % Na_2CO_3	—	75	70	—
31	5	40	A	2	0·2 % Na_2CO_3	—	94	91	—
32	2	20	A	2	Tapwater + 0·5 % soft soap	—	<5	—	—
33	2	20	A	2	Ditto; + 0·1 % Na_2CO_3	—	98	86	72
Series B:					Tapwater; 30° Ca				
34	Mixture A Turkey-red		A	2	+ 0·5 % Agral I	—	64	—	—
35	,, oil		A	2	+ 0·5 % Agral I, 0·05 % Na_2CO_3	—	69	—	—
36	,, 70/100 c.c.		A	2	+ 0·5 % Agral I, 0·1 % Na_2CO_3	—	66	—	—
37	,,		B	2	+ 0·5 % Agral I	—	80	—	—
38	Mixture B		B	2	+ 0·5 % Agral I	—	44	—	—
39	,,		B	2	+ 0·5 % Agral I, 100° Mg	—	44	—	—
40	Mixture A		B	2	+ 0·5 % Agral I, 100° Mg	—	28	—	—

* Intermittent shaking for 50 minutes.

Method of preparing emulsion, and the effect of the presence of ammonia in oil. The aqueous solutions were prepared by diluting a stock solution of sodium carbonate and Agral I (in tap-water) ten times with tap-water, the final concentrations being 0·1 and 0·5 respectively. The emulsions were made up in the following three ways:

Method A. Stock solutions diluted, oil poured into the whole of solution, mixture shaken.

Method B. As A, except that oil was emulsified in 10 per cent. of solution by shaking, then poured into the remaining 90 per cent.

Method C. Oil emulsified in mixtures of the ten times concentrated stock solution, then diluted with tap-water. The period of shaking was from 1 to 3 minutes unless otherwise specified.

With the pyrethrum solution containing 10 per cent. Agral W.B. but no ammonia, method C gave a fairly stable 2 per cent. oil emulsion which contained 64 per cent. of the added oil after 15 minutes, but the emulsions prepared by methods A and B broke almost immediately (Table X, nos. 1–7). A comparison of tests 1 and 2 shows that shaking for a longer time with intervals of rest gave more stable emulsions. The

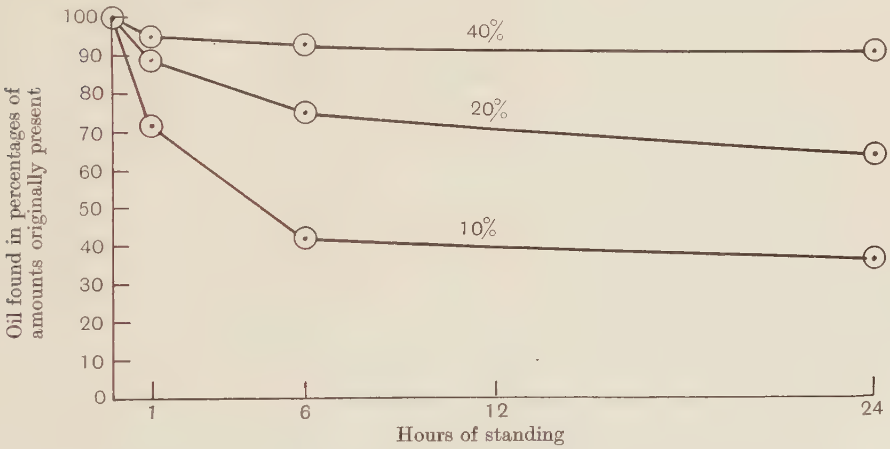

Diagram 1. Stability of emulsions—effect of time of standing. The emulsions contained originally 2 % petroleum spirit extract of pyrethrum and varying proportions of Agral W.B. (ammoniated).

question of shaking was not investigated further, as it was found that addition of small amounts of ammonia to the pyrethrum-Agral solutions facilitated emulsification.

With a pyrethrum solution containing 10 per cent. Agral and 2 per cent. (by volume) of ammonia (S.G. 0·9), method A gave the most stable emulsion. Taking as a basis of comparison the percentage of the added oil found in suspension after 1 hour, the presence of the ammonia is seen to raise the figure from 3 per cent. to 72 per cent. for method A and from 6 per cent. to 44 per cent. for method B, but to leave it unchanged for method C (Table X, nos. 10–12). We can offer no explanation of the fact that methods B and C give less stable emulsions with ammoniated pyrethrum solutions than does method A.

The action of the ammonia is probably as follows. On mixing with water the ammonia in the oil will pass into the water and produce a high alkalinity at the interface for a very short time. It has been shown by Hobson (*loc. cit.*) that the interfacial tension with pyrethrum solutions in petroleum fall with rise in *p*H; with the actual solutions used in these tests the conditions were such that the interfacial tension is immeasurably small. It is not impossible, however, that by further increasing the alkalinity of the aqueous phase the tension may continue to decrease; that is the curve relating the tension to the *p*H becomes asymptotic as

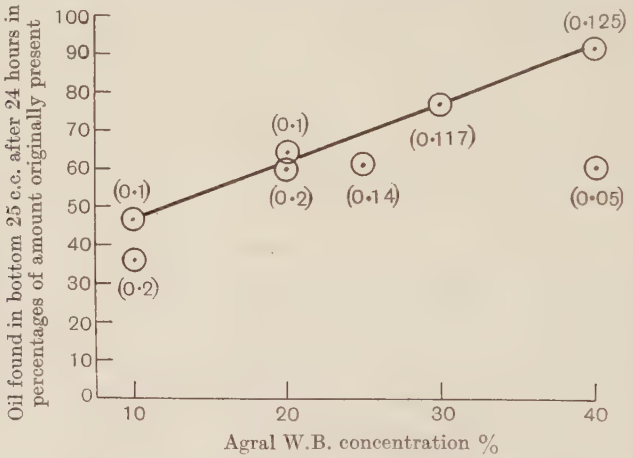

Diagram 2. Relation between Agral W.B. and ammonia contents of petroleum spirit extracts of pyrethrum and the stability of their emulsions.

Dispersion medium—tap water of 30° Ca hardness containing 0·5 % Agral I and 0·1 % Na_2CO_3.

Figures in brackets indicate ratio $\frac{0\cdot 9 \text{ ammonia c.c.}}{\text{Agral W.B. gm.}}$.

the tension approaches zero. If this be so, it can be readily understood how the ammonia assists the oil to emulsify in the first place, without changing significantly the stability of the resulting emulsion, as the final concentration of the ammonia is very low.

Effect of ammonia and Agral W.B. concentration on the stability of the emulsion. The results given in Table X, nos. 9–20, show that increasing the concentration of Agral W.B. in the oil improves the stability of the resulting emulsion increases, provided the ratio of ammonia to Agral W.B. lies within certain limits. The data are insufficient to determine whether there is an actual optimum ratio, but this conclusion might be drawn from Diagram 2, in which the percentage of oil remaining in

emulsion after 24 hours is plotted against the percentage of Agral W.B. in the pyrethrum solution, the numbers in brackets by each point representing the ratio ammonia c.c./Agral W.B. gm. This diagram shows that the points which correspond to ratios between the limits of 0·1 and 0·125 lie along a straight line, the values for ratios outside these limits falling below the line. Where the ratio is less than 0·1, the amount of available ammonia is probably insufficient to disperse the oil at the moment of emulsification. At ratios higher than 0·125 there is some evidence of the stability falling off; this may be a viscosity effect, for as the ratio of ammonia to Agral W.B. increases, the viscosity becomes higher and the initial mixing of oil and water more difficult. Violent shaking effects this in laboratory tests but under field conditions it might prove difficult to mix certain of these more viscous solutions with water.

Gradient of separation of emulsions. To obtain further information on the separation of these mixtures, we decided to do a more elaborate series of tests and determine the proportion of oil found at different levels after the emulsions, prepared from water-miscible extracts, had stood 24 hours. The pyrethrum-miscible oils contained varying amounts of ammoniated Agral W.B., the ratio of ammonia to Agral W.B. being kept at 0·1. The data given in Table X are for very drastic conditions—the bottom levels from a conical funnel being taken off and the oil determined. This procedure not only gave no information as to how the concentration of the oil varied with depth, but actual experiment indicated that the shape of the funnel expedited separation. It was, therefore, decided to carry out certain experiments in a cylindrical vessel.

A straight tube of even bore throughout, every 5 cm. of which corresponded to 25 c.c. volume, was fitted with a rubber stopper holding a glass-tap. The rubber stopper and tube above the tap was covered with mercury, to prevent the emulsion coming into contact with the rubber and from running in the narrower tube of the stop-cock. Water-miscible pyrethrum extracts containing 5, 10, 20 and 40 per cent. of ammoniated Agral W.B., ratio $\frac{\text{ammonia (0·9)}}{\text{Agral W.B.}} = 0·1$, were prepared, and 2 per cent. oil emulsions were made by pouring each into a cylinder containing a measured quantity of a solution of 0·5 per cent. Agral I and 0·1 per cent. sodium carbonate in tap-water (Ca hardness 30°). Each was shaken for half a minute and poured into the separation tube. After standing 24 hours, the mercury above the upper tube of the stop-cock was gently

run out and each 25 c.c. from the bottom of the emulsion to the level of the cream very gently run off. These were shaken and 20 c.c. used for the determination of the oil by the method already described. Although no great accuracy is claimed for this method, the fractions run off, approximate in composition to the successive layers in the tube, provided the flow is sufficiently slow. (The gradations in turbidity were noticed even in the cylinders into which the emulsions were run.) There is some difficulty in shaking in exactly the same way in every case, but we believe the results on the whole to be comparable with each other.

Table XI. *Degree of separation of pyrethrum petroleum spirit emulsions containing ammoniated Agral W.B.*

Ratio $\frac{0\cdot9 \text{ ammonia c.c.}}{\text{Agral W.B. gm.}}=0\cdot1.$

Dispersion medium: Ca-hard water 30° total hardness containing 0·5 % Agral I and 0·1 % Na_2CO_3.

Height in cm.	Mean amount of oil found after 24 hours in each 5 cm. of height in percentages of original amount present			
	5 % ammoniated Agral W.B.	10 % ammoniated Agral W.B.	20 % ammoniated Agral W.B.	40 % ammoniated Agral W.B.
0–5	10	51	89	96·1
5–10	22·7	73·4	97·5	97·7
10–15	46·1	81·3	103·1	97·7
15–20	60·9	84·4	103·1	98·4
20–25	74·2	87·5	104·7	98·4
25–30	78·1	88·4	105·5	98·4
30–35	82·8	89·0	104·7	—
35–40	89·0	92·2	105·5	98·4
40–45	90·6	95·3	105·5	99·2
45–50	94·5	96	105·5	99·2
50–55	94·5	96	105·5	99·2
55–60	Much cream—separated clear oil	Some cream	A little cream	103

Note. Each water-miscible extract on cooling to 0° C. gave a small solid deposit.

The data are set out in Table XI and graphed in Diagram 3. They express the mean amount of oil found for each 5 cm. of height from the bottom to the level of the cream. The data in Table XI and the curves of Diagram 3, confirm that the stability rises as the amount of ammoniated Agral W.B. is increased in the water-miscible extract, since the average gradient for the change of oil concentration with height becomes less and less steep as one passes from the 5 per cent. to the 40 per cent. mixture. There was no visible creaming in the 40 per cent. mixture, whereas the 5 per cent. not only creamed but to a large extent separated out clear oil on the surface. In the layers just below the cream

the concentration of oil in all the mixtures was approximately the same and the gradients all become very flat. The 40 per cent. mixture obviously gives an emulsion approximating to complete stability; there has, however, been a slight separation, for although no visible creaming was noted, the gradient is slightly steeper at the very beginning and the end of the curve. The curve for the 5 per cent. mixture is sigmoid in shape, due to the nature of distribution of globule size. The data and gradients of separation indicate that the emulsions prepared from the water-

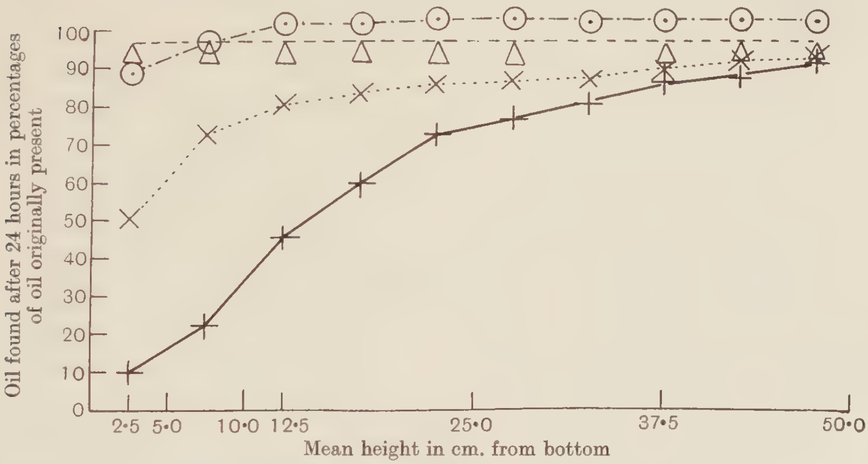

Diagram 3. Permanence of emulsions prepared from Agral W.B. and pyrethrum petroleum spirit extracts.

Mean percentages of oil found in each 5 cm. of height from bottom, after 24 hours.

+ +	Miscible oil contains	5 %	ammoniated Agral W.B.
× ×	,,	10 %	,,
⊙ ⊙	,,	20 %	,,
△ △	,,	40 %	,,

In each case 6 c.c. of the miscible oil was shaken for half a minute with water of 30° total calcium hardness which contained in solution 0·5 % Agral I and 0·1 % Na_2CO_3.

miscible extracts containing 10 and 20 per cent. of ammoniated Agral W.B. in the way described should be stable enough for application as sprays on a large scale. This would be particularly true if the tanks were stirred. With respect to the 5 per cent. mixture, no expression of opinion as to its utility is possible until further information is forthcoming as to the relative effectiveness of quick-breaking and stable emulsions.

The effect of different concentrations of calcium and magnesium salts. By adding magnesium and calcium sulphates to Harpenden tap-water artificial waters of varying degrees of hardness were obtained. The results with magnesium present are shown in Table X, nos. 21–24. Experiments

were made with waters containing from 0 to 100° hardness as magnesium and 0·1 per cent. Na_2CO_3 and a pyrethrum solution containing 40 per cent. Agral W.B. and 5 per cent. ammonia was used. The results, which are also illustrated in Diagram 4, show that the stability rapidly fell with increasing magnesium concentration. Although the *p*H may have changed by adding magnesium sulphate, this probably did not influence the results appreciably, as it was found with the highest concentration of magnesium (100° hardness) that doubling and trebling the concentration of sodium carbonate had little effect on the results (Table X, nos. 24 and 26, 27 and 28).

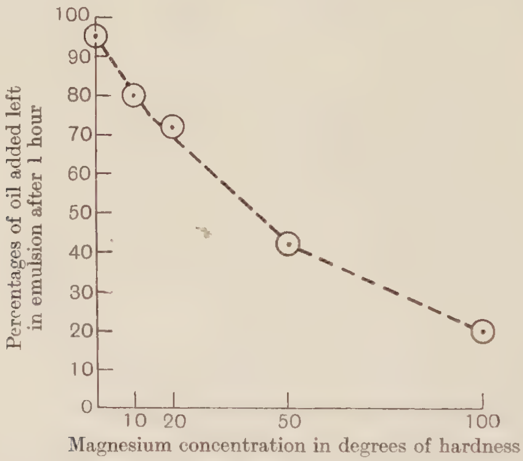

Diagram 4. The effect of magnesium on the separation of emulsions.

Tap-water of 30° total calcium hardness containing 0·1 % Na_2CO_3 and increasing amounts of magnesium sulphate.

Increasing the calcium hardness of the tap-water to 100° was without effect on the stability provided the sodium carbonate was increased from 0·1 per cent. to 0·2 per cent.; this can be seen by comparing tests nos. 17, 30 and 31, Table X. With water having 100° hardness as calcium the amount of oil remaining in suspension after 1 hour was 75 per cent. with 0·1 per cent. of added sodium carbonate and 94 per cent. with 0·2 per cent., the value for water containing 30° hardness and 0·1 per cent. sodium being 95 per cent. This result was not unexpected as the added calcium sulphate must have decreased the hydroxyl ion concentration and increased the amount of calcium in solution, conditions which have been shown by Hobson to increase their interfacial tension.

Miscellaneous results. By adding 0·1 per cent. sodium hydroxide to water containing 100° hardness as magnesium, an excellent emulsion was at first obtained, but a precipitate of magnesia separated and carried the oil to the surface. As magnesium oxalate is relatively little ionised, the effect of adding ammonium oxalate was tried and found of no value. These results are shown in Table X, nos. 25, 29.

Soft soap in 0·5 per cent. concentration failed to replace the mixture of 0·1 per cent. sodium carbonate and 0·5 per cent. Agral I added to the tap-water (Table X of 32, 15). A mixture of 0·5 per cent. soft soap and 0·1 per cent. sodium carbonate was more effective in emulsifying than 0·5 per cent. Agral I and 0·1 per cent. sodium carbonate (Table X and 33, 15), but it must be remembered that the former mixture renders the solution more alkaline.

Turkey-red oil preparations. The samples used for testing the stability of their emulsions in water were those employed for toxicity tests, and their preparation is described on p. 221. The Gerber-tube method is hardly so satisfactory with these preparations owing to the rather great solubility of turkey-red oil in water—but it was thought advisable to carry out strictly comparative tests for both calcium and magnesium-hard waters. Preliminary data for samples A and B (p. 221) are given in Table X, nos. 34–40.

Mixtures A and B (p. 221) prepared from neutral turkey-red oil (with no addition of oleic acid) are noticeable for the ease with which they mix with hard waters of both types and the readiness with which by gentle stirring they can be kept thoroughly incorporated with water. When oleic acid is used to neutralise the alkalinity, characteristic of commercial turkey-red oil, this no longer holds, and the addition of alkali to the aqueous phase is requisite. Figures showing the gradient of separation for mixtures A and C (p. 221) for various types of dispersion media are given in Table XII.

For mixture A (p. 221) (set out under series B in Table XII), the permanence of the emulsion for various types of water is shown by these figures to be quite high and the addition of materials to the dispersion medium for purposes of lowering surface tension is not requisite.

For mixture C (p. 221) (set out under series A in Table XII) the ease of mixing and the permanence of the resulting emulsion is considerably modified by the addition to the water of sodium carbonate or of some material lowering surface tension. In the case of hard water of the type used in these experiments (26° temporary hardness) the addition of 0·1 per cent. sodium carbonate made little difference to the

stability of the emulsion, but a further addition of 0·5 per cent. Agral I to the water phase greatly added to the stability. Greater permanence could also be achieved by increasing the proportion of sodium carbonate to 0·175 per cent., and by a further increase to 0·25 per cent. almost complete stability was established, though this would hardly be required in practice.

Table XII. *Degree of separation of pyrethrum petroleum spirit emulsions containing turkey-red oil.*

Mean amount of oil found after 24 hours in each 5 cm. of height from bottom expressed in terms of the amount found immediately after mixing *in percentages*. 6 c.c. of miscible oil shaken well with 295 c.c. of aqueous medium for 30 seconds.

Series A. Miscible oil contains alkaline turkey-red oil, oleic acid, petroleum spirit (Mixture C, p. 221).

Series B. Miscible oil contains neutral turkey-red oil, petroleum spirit (Mixture A, p. 221).

The tap water used had a temporary hardness of 26°, total hardness 30°.

	Dispersion media						
Height in cm.	Tap water	Tap water + 0·1 % Na_2CO_3	Tap water + 0·1 % Na_2CO_3 0·5 % Agral I	Tap water + 0·175 % Na_2CO_3	Tap water + 0·25 % Na_2CO_3	Water of Ca hardness 30°, Mg hardness 30° +0·25 % Na_2CO_3	Water of Ca hardness 30°, Mg hardness 30° 0·5 % Agral I
Series A:							
0–5	1·6	3·2	52	13·2	82·35	22·1	—
5–10	3·2	3·2	63·7	32·1	94·1	29·1	—
10–15	3·2	4·8	71·2	41·5	101·9	36·3	—
15–20	4·8	—	76·8	50·1	—	40·0	—
20–25	4·8	4·8	82·3	58·5	104·9	43·6	—
25–30	4·8	4·8	85·1	61·3	106·9	44·6	—
30–35	4·8	6·4	87·6	66·1	106·9	46·3	—
35–40	6·4	8·0	91·6	69·7	106·9	48·2	—
40–45	6·4	6·4	92·8	73·6	106·9	51·8	—
45–50	6·4	8·0	93·4	73·6	109·8	51	—
50–	6·4	—	—	—	116·6	This mixture reverted to water-in-oil type	—
			Mean of two determinations				
Series B:			(0·5 % Agral I No Na_2CO_3)			(No Na_2CO_3)	
0–5	75	—	100	—	—	89·2	90·2
5–10	80	—	,,	—	—	91·9	91·3
10–15	83·7	—	,,	—	—	94·6	96·7
15–20	87·5	—	,,	—	—	96	96·7
20–25	85	—	,,	—	—	96	98·9
25–30	90	—	,,	—	—	96	98·9
30–35	90	—	,,	—	—	96	100
35–40	90	—	,,	—	—	96	101
40–45	88·7	—	,,	—	—	96	101
45–50	90	—	,,	—	—	96	101
50–	90	—	,,	—	—	100	101

The admixture of oleic acid with turkey-red oil enables a larger amount of petroleum spirit to be incorporated with the miscible oil. It has, however, the disadvantage in mixture C (p. 221) of rendering the resulting emulsion quite unsuitable in magnesium-hard waters, the water-in-oil type being formed. This effect is in marked contradistinction to the miscible oils prepared from pure turkey-red oil (mixture A, series B, Table XII), which showed an almost complete stability for 24 hours in water of 30° calcium and 30° magnesium hardness.

The results in Table XII are plotted in Diagram 5.

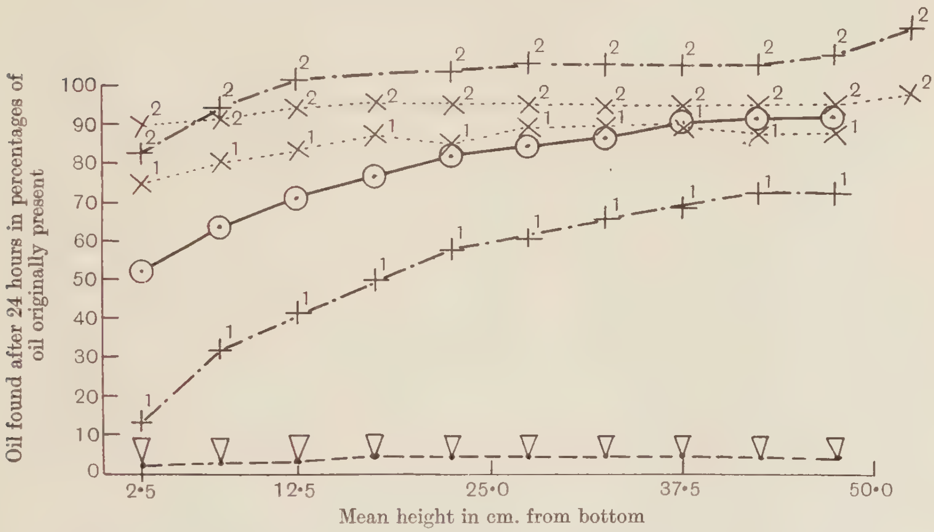

Diagram 5. Permanence of turkey-red oil pyrethrum extract emulsions. Mean percentages of oil in each 5 cm. of height from bottom after 24 hours.

• — — — •	Mixture C (p. 221) in tap water		
▽ — — — ▽	,,	,,	+0·1 % Na_2CO_3
+[1] — • — +[1]	,,	,,	+0·175 % Na_2CO_3
+[2] — • — +[2]	,,	,,	+0·25 % Na_2CO_3
⊙————⊙	,,	,,	+0·1 % Na_2CO_3 + 0·5 % Agral I
×[1] • • • • • • • • ×[1]	Mixture A (p. 221) in tap water		
×[2] • • • • • • • • ×[2]	,,	magnesium-hard water	

DISCUSSION AND CONCLUSIONS.

A water-miscible oil containing an extract of pyrethrum must obey the following conditions:

A. The interfacial tension of the oil, or extract against the aqueous phase must be of a low order, otherwise the resistance to spontaneous emulsification may be too great. This aspect of the problem has been

considered in detail by Hobson (*loc. cit.*). In the case of pyrethrum extracts, the easy dispersion of the oil in the aqueous phase can be obtained by rendering the aqueous phase alkaline, and particularly by the addition to it of materials lowering surface tension, such as soft soap in soft waters, or in calcium-hard waters a reagent such as saponin or the commercial product Agral I, especially if the latter is rendered slightly alkaline. Water-miscible oils in which have been incorporated neutral turkey-red oil (sulphonated castor oil) show this property not only in the case of soft and calcium-hard waters, but also with magnesium waters without the addition to the water phase of materials tending to lower surface tension. A commercial product known as Agral W.B. when incorporated with the miscible oil accentuated the readiness of miscibility, particularly if, wholly or in part, combined with ammonia. Water-miscible oils containing Agral W.B., ammoniated or otherwise, usually require the surface tension of the aqueous phase to be lowered and to be alkaline as indicated above.

B. Readiness of admixture does not necessarily imply that the resulting emulsion is stable. For this purpose a separate stabilising material has to be added, or conditions have to be arranged so that one of the products used in accentuating the miscibility of the oil shall be present in such a form and in such an amount as to secure stability. Such emulsions must have a degree of stability sufficiently great to admit of their ready application with gentle stirring. In the case where Agral W.B. is used our data show that stability is increased by the incorporation with it of a strong ammonia solution in certain proportions. Increase in the amount of ammoniated Agral W.B. to a certain limit tends towards greater stability, a mixture of pyrethrum extract in petroleum spirit to which had been added 10 or 20 gm. per 100 c.c. ammoniated Agral W.B. have a relatively high stability in hard water containing 0·5 per cent. Agral I and 0·1 per cent. sodium carbonate. With magnesium-hard waters there is so great a reversion of phase that many emulsifiers are rendered useless. For such water we have found neutral turkey-red oil mixtures are very suitable. Light petroleum and lubricating oil preparations mix readily with hard water containing alkaline Agral I if 10 per cent. ammoniated Agral W.B. $\frac{0{\cdot}9 \text{ ammonia in c.c.}}{\text{Agral W.B. in gm.}} = 0{\cdot}1$ be incorporated; but some care in balancing the various ingredients is required or the miscible oil may separate out into two phases, particularly on cooling.

C. The proportion of the pyrethrins to solvent must be sufficiently

high to give on dilution a strength requisite to kill the pest and a concentration of oil which shall be low enough to do no damage to foliage. This is a matter of great importance and demands a careful balancing of the mixture. If fixed fatty oils are used in addition to or in the place of petroleum solvents it is probable that greater latitude may be allowed. In the experiments described which only deal with petroleum solvents, the petroleum derivatives rarely if ever exceeded 0·5 per cent. in the strongest diluted spray. A grave damage can be done to foliage by the application of petroleum oils and spirits. Something of the order of 1 per cent. should be fixed as an upper limit for the amount of them allowable in the diluted pyrethrum sprays; actually with smaller quantities than this we have noted an accentuation of the toxicity of pyrethrum, even in cases where the oil used by itself in the same concentration produced little or no lethal effects.

D. The miscible oil mixture must be relatively stable, *i.e.* must not separate out into two liquid phases—or become very turbid on cooling to 0° C. Absolute clarity under these conditions in the case of concentrated pyrethrum preparations is not easy to obtain, and the separation of a small amount of precipitate is not a grave matter, provided it does not lead to the formation of a second liquid phase.

E. The active principles of pyrethrum should be stable in the solution, and they must not suffer such chemical change as to lead to any great loss of toxicity in several months. Our experiments lead us to believe that extracts of pyrethrum made with alcohol (95 per cent.) and petroleum solvents maintain their toxicity over many months in temperate climates or at temperatures of 28° C.; but it is probable that in tropical regions some care in storage would be requisite and that the period should not be too prolonged. In our experiments with extracts containing neutral turkey-red oil or Agral W. B. in concentrations up to 20 per cent. the storage properties were relatively good, and our data, so far, indicate that the combination of a certain proportion of ammonia with the Agral W.B. for purposes of improving the emulsification properties has no deleterious action on toxicity in a period of some months.

It must, however, be noted that the water-miscible petroleum extracts of pyrethrum were not found to be superior to extracts of pyrethrum made with 95 per cent. alcohol either in the degree of permanence of their toxicity or in the stability of their emulsion in water.

We desire to express our thanks to Mr C. T. Gimingham for help and advice during the course of this investigation, and to Mr A. Oggelsby

and Miss I. Randall for repeated help in carrying out the experiments. Our thanks are also due to the Anglo-Persian Oil Co., Ltd., and to Imperial Chemical Industries for the provision of certain of the materials used in this investigation.

Summary.

1. Pyrethrum flowers (*Chrysanthemum cinerariaefolium*) both as whole heads and as powder retain their insecticidal properties at ordinary temperatures and at 28° C. for considerable periods if stored in closed vessels. If exposed to the atmosphere in a thin layer as finely ground powder there is risk of loss of toxicity.

2. Alcohol and petroleum extracts of pyrethrum retain their toxicity in temperate climates over many months. Alcohol extracts readily give permanent emulsions when added to water; petroleum extracts require the incorporation of an emulsifier.

3. Water-miscible petroleum extracts of pyrethrum can be prepared by the addition ofcertain materials, such as ammoniated Agral W.B. and neutral turkey-red oil.

4. A study has been made of the degree of permanence of the active principles in alcoholic and water-miscible petroleum extracts at ordinary British temperatures and at 28° C. and also in emulsions of these extracts in alkaline spray fluids of varying *p*H. The active principles proved more permanent than has been usually supposed.

5. The readiness with which water-miscible petroleum extracts disperse in the aqueous phase and the stability of the emulsions formed under a variety of conditions have been investigated.

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THE BIOLOGICAL DECOMPOSITION OF PLANT MATERIALS

PART IV. THE BIOCHEMICAL ACTIVITIES ON STRAWS OF SOME CELLULOSE-DECOMPOSING FUNGI

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(With 2 Text-figures.)

CONTENTS.

I. Introduction.

Certain biochemical aspects of the decomposition of straws by a general microflora were discussed in the second communication of this series (Norman (5)). It was shown that in a normal aerobic decomposition in the presence of a sufficiency of available nitrogen, the main constituent to be decomposed was the cellulose. This has also been clearly demonstrated by Waksman (10), and cellulose must in consequence be viewed as being readily assimilable. Many organisms of various types have from time to time been described as cellulose-decomposers, but few of these may validly be described as active under pure cultural conditions. It

may be, as has repeatedly been suggested, that certain of them are most efficient when in association with a secondary microflora. But nevertheless, the technique commonly employed for testing cellulose-decomposing ability is too critical, in that it shows not those organisms that are capable of utilising cellulose as a source of energy but only those which can utilise cellulose *alone* as the sole source of energy both for growth and maintenance. It is true that some organisms, such as *Spirochaeta cytophaga*, have been obtained, which can utilise only cellulose, and are adversely affected by the presence of sugars or other carbohydrate sources. But it may equally be true that there are others which, though unable to utilise cellulose alone to any extent, are able to assimilate it when they are developing actively on some other carbonaceous material. It is probable that this applies in particular to the germination of the spores of certain fungi.

Köning (2) was the first to suggest that fungi are more important than bacteria in the decomposition of cellulosic material in the soil, and the same worker isolated a strain of *Trichoderma* which he found particularly active in this process. McBeth and Scales (3) and Scales (7) tested a number of fungi, including many common *Aspergilli* and *Penicillia*, and found them to be capable of utilising cellulose.

It has been shown by Sée (8) that fungi will develop on paper, and by Bright, Morris and Summers (1) that the mildewing of raw cotton and cotton fabrics is due to the action of common fungi. There is no doubt that the attack may be assisted by the presence of available carbohydrate material in the size often employed and Thaysen and Bunker (9) point out that the mildewing flora is often extended for this reason. It has therefore been demonstrated that many fungi are capable of utilising cellulose, and Waksman (11) and others are of the opinion that they are mainly responsible for this process in the soil.

Scheme of work.

Since it seemed clear that the fungi have an important rôle in the decomposition processes, a number were isolated from rapidly decomposing straw with a view to following their activities in detail. Certain physiological characteristics of a number of these organisms were described in a recent paper (Norman (6)). Their biochemical activities in pure culture on natural materials are described below, and contrasted with decompositions effected by a mixed microflora at various temperatures. The extent of nitrogen immobilisation in relation to carbohydrate decomposed has also received attention.

II. Experimental.

Technique.

In the first series of experiments decomposition of sterile straw was effected by pure cultures of various fungi. The following organisms were tested: *Trichoderma* sp., *Phoma* sp., *Aspergillus flavipes*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. terreus*, *A. versicolor* and *Actinomyces* sp.

Weighed samples of oat straw were bottled and sterilised by treatment at 115° C. for half an hour on four consecutive days. This treatment is admittedly rather drastic, but it is necessary for complete sterilisation. Available nitrogen was added to each as sterile ammonium carbonate at the rate of 1 gm. nitrogen per 100 gm. dry straw. This is slightly in excess of the theoretical final requirement which is about 0·8 gm. per 100 gm. straw. A heavy inoculum of a suspension of the spores of the required organism in sterile water was added to each, the total volume of water and ammonium solution being adjusted so that the moisture content of the straw was 70 per cent. The bottles were laid on their sides in an incubator at 30° C., and rotated frequently during the first few days so that wetting of the straw might be uniform. The period of decomposition was 48 days, since preliminary experiments showed that in pure culture the losses of organic matter after this time were very small. Platings were made at the end of the experiments to ensure that chance infection had not taken place. Fungal contamination, when it occurred, was readily noticeable, since the straw was characteristically coloured by the spores and mycelium of each of the organisms.

For purposes of comparison a second series of decompositions was carried out with a mixed soil flora, under similar conditions, and bottles were incubated at 20°, 30°, 35° and 50° C.

Analyses.

In both series the following determinations were carried out on the rotted material: (i) dry matter; (ii) ammoniacal nitrogen; (iii) total nitrogen; and on the dried material: (iv) total furfuraldehyde yield; (v) cellulose (Cross and Bevan); (vi) furfuraldehyde from Cross and Bevan cellulose product; (vii) carbon dioxide yield from uronic acids. The methods employed in the latter determination were described in the first paper of this series (Norman (4)). By calculation from them can be obtained detailed information as to the losses of the three chief groups of assimilable carbohydrate material in the straw, the fourth major

constituent, lignin, being unavailable. The basis of these calculations was given in considerable detail in the paper referred to above, but in view of their importance, it is proposed briefly to summarise them here.

(1) *Carbohydrate constituents.*

Interpretation of analyses. In any mature plant tissue, the three major groups of biologically available material are the cellulose, the polysaccharide intimately associated with the cellulose, and the hemicelluloses (or polyuronides, as they may more suitably be termed). If a cellulose determination be carried out by the Cross and Bevan chlorination method, the resulting produce consists of "pure" cellulose, together with an associated polysaccharide. In general this is found to be built up of pentose units in the form of an anhydro-xylose compound, or xylan, though in certain woods a hexosan, usually mannan, is also found. In oat straw this associated polysaccharide is undoubtedly a xylan. The steric similarity between the glucose units of cellobiose in cellulose, and the xylose units of xylan, together with the extreme resistance of the xylan to extraction, makes it probable that it has been laid down during development in the closest physical association with the cellulose, and probably by the same mechanism. All pentose units on distillation with 12 per cent. HCl yield furfuraldehyde, and the xylan in the cellulose may accordingly be determined in this way. By subtraction from the Cross and Bevan cellulose, a figure may be obtained for "pure" cellulose. Actually it is not of pre-eminent importance to determine the "pure" cellulose content, since in view of its intimate association with the xylan, it is commonly found that the decomposition of each occurs in parallel. It is patent that, if the cellulose is unattacked, only that portion of the xylan which is superficial to the cellulose can be decomposed. The significance of having a figure for pentose units associated with cellulose lies chiefly in the assay of the hemicelluloses. It is impossible to determine this group of substances directly owing to their constitutional heterogeneity even in a particular plant tissue; it is only possible to estimate certain units. The hemicelluloses are commonly built up of hexose, uronic acid, and pentose units, though sometimes only two of these may be present. The wide distribution of the uronic acids—either galacturonic or glucuronic—in this class of substance is only now becoming recognised, and is the justification for the new term "polyuronide" for a polysaccharide containing uronic groups. Furfuraldehyde is given on distillation from the pentose and uronic residues, and carbon

dioxide from the carboxyl group of the uronic acids alone[1]. It is clear that by difference a figure may be obtained for the pentose groups in the hemicelluloses, the furfuraldehyde from the xylan in the cellulose plus that from the uronic acids, being subtracted from the total furfuraldehyde yield of the tissue. Information is thus obtained as to the content of pentose and uronic acid in the hemicellulose, but is wanting as regards the hexose units. If the hemicelluloses are actually extracted from the tissue and the percentage yield of furfuraldehyde from the pentose groups of the preparation determined, then a factor may be obtained for that particular tissue. The use of such a factor on decomposing materials is, however, quite indefensible, for it is impossible to regard the various units involved in the hemicelluloses as of equal availability or equal biological value. Without making an extraction or re-determining the factor at every stage—a most laborious procedure and one involving the use of a considerable bulk of material—it is only possible to follow accurately the losses of pentose and uronic acid from the hemicelluloses. In the case of the organisms employed in this work it has been shown that, in general, the hexoses are rather more suitable than their sterically related pentosans and it is reasonable to suppose that, when a heavy loss of pentose occurs, the hexoses are also removed. It is also true that the loss of pentose from the hemicelluloses may be masked by the production, presumably from cellulose, of furfuraldehyde yielding substances. There is as yet no method by which it is possible to distinguish between original pentose and biologically formed pentose.

Analytical figures. The straw employed in this work was that used for the mixed flora decompositions. A complete analysis was given in the first part of the series (Norman (4)). The chief constituents of importance here are:

	%
"Pure" cellulose	43·8
Xylan associated with cellulose	9·3
Hemicelluloses...	22·8
Lignin	18·5

The results of analyses of the straw decomposed by pure cultures of various organisms are given in Table I, and of the mixed flora decompositions at various temperatures in Table II.

[1] The small amount of pectin present in a mature tissue such as straw (1 per cent.) is not accounted for separately but is treated here as a polyuronide, which in fact it is. For separate treatment see Norman (5).

Table I. *Decomposition of oat straw by various fungi at 30° C. for 48 days.*

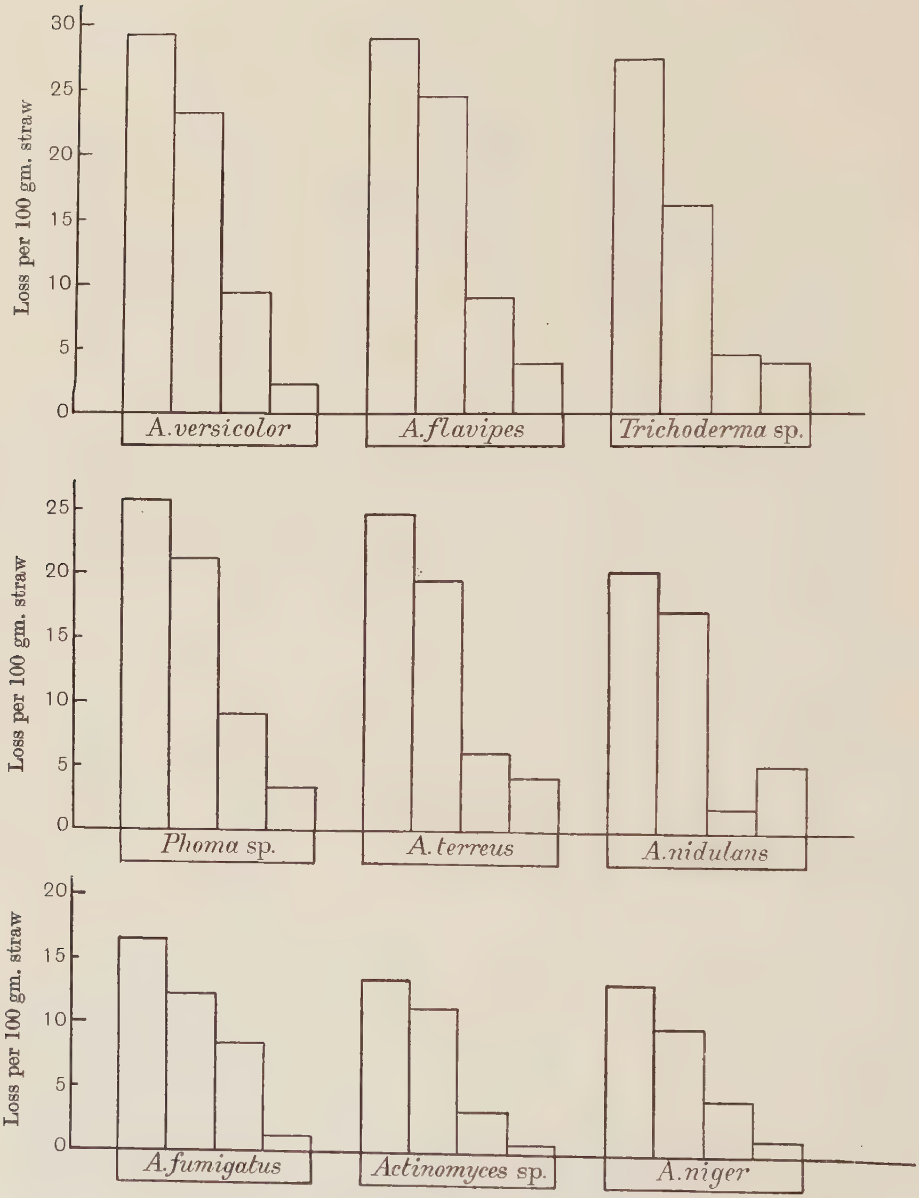
Organism	Loss of dry matter	Total furfuraldehyde yield	C. and B. cellulose	Furfuraldehyde yield from C. and B. cellulose	CO_2 yield
A. versicolor	29·29	11·57	38·97	16·06	1·22
A. flavipes	28·94	10·49	35·06	14·00	1·22
Trichoderma sp.	27·10	14·13	45·55	14·41	0·91
Phoma sp.	25·54	10·52	38·54	13·50	1·01
A. terreus	24·87	12·20	39·40	15·21	1·01
A. nidulans	20·29	14·40	38·81	10·72	1·41
A. fumigatus	16·67	11·60	47·47	15·95	1·00
Actinomyces sp.	13·46	15·53	47·32	15·35	1·38
A. niger	13·34	14·25	48·65	14·41	0·93

For purposes of comparison the figures just recorded must be recalculated on a basis of 100 gm. original dry straw, and interpreted in terms of cellulose, xylan associated with cellulose, and pentose in hemicelluloses as described above. When this is done the differences in specific activity between the organisms stand out clearly. In Table III and Fig. 1 will be found the pure culture decompositions and those with a mixed flora at various temperatures in Table IV and Fig. 2.

Table II. *Decomposition of oat straw by mixed flora at various temperatures for 48 days.*

Temp. ° C.	Loss of dry matter	Total furfuraldehyde yield	C. and B. cellulose	Furfuraldehyde from C. and B. cellulose	CO_2 yield
20	38·85	7·35	31·49	14·02	1·33
30	51·00	5·79	30·16	13·55	1·35
35	50·56	7·41	25·94	10·91	1·58
50	42·14	7·02	26·50	10·22	1·79

Discussion of results. The more efficient of the organisms tested bring about an apparent loss of 25–30 per cent. of the straw, the major portion of this being accounted for by loss of cellulose. The hemicelluloses, as assessed from the loss of pentose units, suffer extensive losses, but the bulk is less than the lost cellulose. Perhaps the most striking thing is that the organisms seem in general to be so similar in their relative abilities to decompose the carbohydrate constituents. Each group seems to be attacked by all the organisms to an extent relatively proportional to the total loss of organic matter and there is no organism which fails completely to attack any one group. The differences merely seem to be of degree with perhaps two exceptions, to which attention may be drawn. In the decomposition effected by *A. nidulans*, neither the uronic



Time in each case—48 days. Temperature 30° C.

Fig. 1. Decomposition of oat straw by pure cultures of fungi.
1st column = Organic matter. 2nd column = Cellulose. 3rd column = Pentose in hemicelluloses. 4th column = Xylan in cellulose.

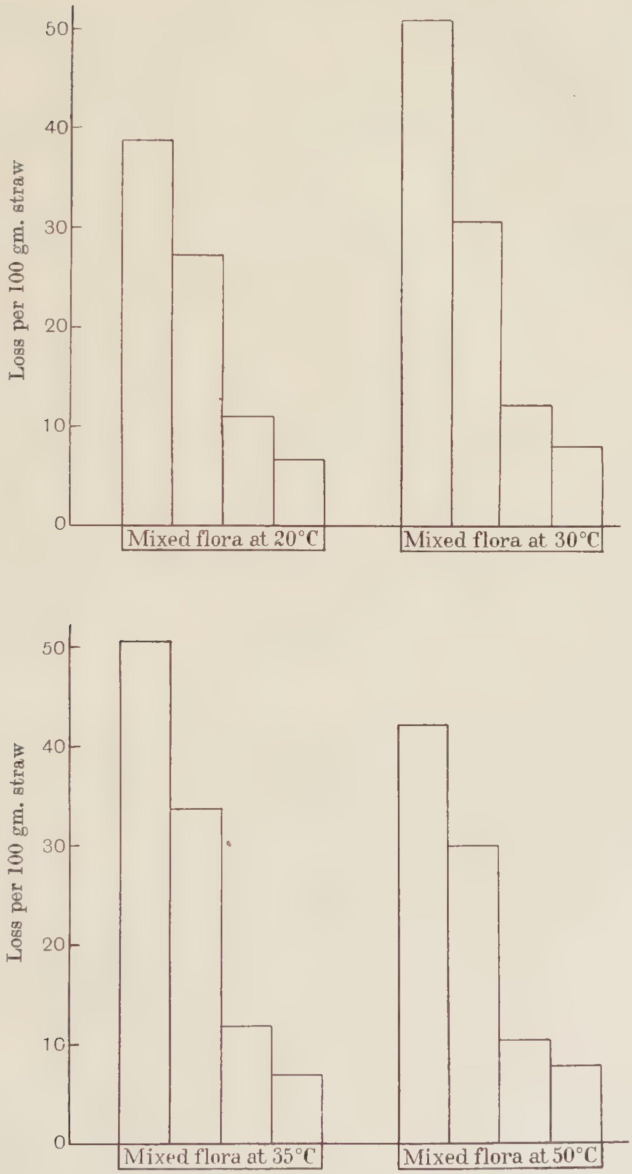


Fig. 2. Decomposition of oat straw by mixed floras.

acid nor the pentose units of the hemicelluloses suffer more than a slight loss, and it would rather seem as though cellulose is more suitable for this organism. The xylan associated with the cellulose, though pentose in nature, seems readily to be removed. In the case of *A. fumigatus* the position is reversed, for there the loss of hemicelluloses appears to be heavy, and probably greater in extent than the loss of cellulose.

Table III. *Decomposition of oat straw by various fungi at* 30° *C. for* 48 *days.* (*Expressed on* 100 *gm. original dry straw.*)

Organism	Dry matter	Total furfuraldehyde yield	C. and B. cellulose	Furfuraldehyde from C. and B. cellulose	CO_2 yield	Uronic acid anhydride	Furfuraldehyde from uronic anhydride	Furfuraldehyde from pentose groups in hemicelluloses	Anhydro-pentose units in hemicelluloses	Xylan associated with C. and B. cellulose	Pure cellulose
—	100	16·08	53·14	5·99	1·21	4·84	0·81	9·30	14·4	9·3	43·9
Aspergillus versicolor	70·71	8·18	27·55	4·42	0·86	3·44	0·57	3·12	5·0	6·9	20·7
A. flavipes	71·06	7·45	24·92	3·49	0·87	3·48	0·58	3·34	5·3	5·4	19·5
Trichoderma sp.	72·90	10·30	33·21	3·48	0·66	2·64	0·44	6·40	9·9	5·4	27·8
Phoma sp.	74·46	7·83	28·71	3·87	0·75	3·00	0·50	3·45	5·4	6·0	22·7
Aspergillus terreus	75·13	9·16	29·60	3·38	0·76	3·04	0·51	5·30	8·2	5·2	24·4
A. nidulans	79·71	11·48	30·94	2·64	1·12	4·48	0·75	8·10	12·5	4·1	26·8
A. fumigatus	83·33	9·67	39·56	5·25	0·83	3·32	0·55	3·90	6·0	8·1	31·5
Actinomyces sp.	86·54	13·44	40·96	5·44	1·19	4·76	0·79	7·20	11·1	8·4	32·6
Aspergillus niger	86·66	12·35	42·15	5·26	0·81	3·24	0·54	6·60	10·2	8·1	34·1

The decomposition effected by a mixed flora is considerably greater in extent than that by any individual organism and is greater at 30° C. and 35° C. than at 20° C. or 50° C. as might be expected. That there is a loss of over 40 per cent. in a straw incubated at 50° C. is rather remarkable and is clear evidence of the high activity of organisms at that temperature, and of the wide nature of the flora present. It is clear that, within reasonable limits whatever the temperature may be, there are organisms present capable of effecting an extensive decomposition.

Table IV. *Decomposition of oat straw by mixed flora at various temperatures for* 48 *days.* (*Expressed on* 100 *gm. original dry straw.*)

Temp. ° C.	Dry matter	Total furfuraldehyde yield	C. and B. cellulose	Furfuraldehyde from C. and B. cellulose	CO_2 yield	Uronic acid anhydride	Furfuraldehyde from uronic anhydride	Furfuraldehyde from pentose groups in hemicelluloses	Anhydro-pentose units in hemicelluloses	Xylan associated with C. and B. cellulose	Pure cellulose
—	100	16·08	53·14	5·99	1·21	4·84	0·81	9·3	14·4	9·3	43·9
20	61·15	4·49	19·25	1·71	0·51	3·24	0·54	2·2	3·4	2·7	16·6
30	49·00	2·84	14·79	0·98	0·66	2·64	0·44	1·4	2·2	1·5	13·3
35	49·44	3·66	12·82	1·40	0·78	3·12	0·52	1·7	2·6	2·2	10·6
50	57·86	4·06	15·31	0·91	1·04	4·16	0·69	2·5	3·9	1·4	13·9

A significant fact, clearly visible in Table III and Fig. 1, is that in every case the losses of the various constituents, as given by the analyses, together amount to more than the total loss of organic matter. This apparent anomaly is of easy explanation, being caused mainly by the fact that there is as yet no valid method of estimating the true loss of the organic matter of the straw, and that the figure obtained is the difference between total loss and the microbial tissue synthesised. From the nitrogenous figures to be presented later it is possible to form a rough estimate of the amount of tissue built up. It will be shown that the organisms in attacking the straw convert to organic nitrogen 0·7–0·8 gm. of inorganic nitrogen for every 100 gm. original dry straw. Fungal tissue may contain from 2 to 6 per cent. nitrogen, but in general the figure is found to be 4–5 per cent. On this basis approximately 15 per cent. of the rotted material would be accounted for by fungal tissue, and the figure obtained for loss of organic matter would be correspondingly too low. When bacteria are concerned, as in a mixed flora, the synthesised microbial tissue is distinctly less in amount, since bacterial cells commonly contain 10 per cent. or more of nitrogen as protein.

To a much smaller degree the apparent discrepancy in the analytical figures may be caused by the accumulation of intermediary products in degradation.

It is not justifiable to suppose that when cellulose is so attacked that it no longer is estimated as cellulose, it must of necessity have been reduced to carbon dioxide and water only. It seems more likely that the hemicellulose sugars when liberated by enzymic hydrolysis, may so be disposed of. If the carbon dioxide evolution of a rapidly decomposing straw be measured it is found that a peak occurs quite early coinciding in fact with the rapid initial loss of hemicelluloses, and after that point the evolution falls off considerably even though analyses show that cellulose is very rapidly being lost. It would appear that the process of degradation of cellulose to carbon dioxide is considerably slower than that of hemicellulose to carbon dioxide. Some evidence as to possible intermediary stages in the cellulosic degradation were given in Part II of this series (Norman (5)), when it was shown that there was an actual production of furfuraldehyde yielding material, presumably removed as the rate of decomposition lessens. Any such accumulation would tend to mask the true loss of pentose units from the hemicelluloses, and it is possible that this actually occurs in some or all of the analyses presented.

(2) *Nitrogen immobilisation.*

Analytical figures. At the end of the period of decomposition, both the total nitrogen content, and the free ammonia of the straws was determined so that a figure might be obtained for the nitrogen utilised by the organisms in building up microbial protein. In the first column of Tables V and VI will be found the difference figure for organic nitrogen in the rotted material. Only a portion of this organic nitrogen has, however, been synthesised from the added ammonia, the remainder originating in the plant proteins of the straw. Unless they are wholly unavailable, a very unlikely supposition, they will have been, in part at least, utilised by the organisms. There is no means of distinguishing between the nitrogen of unattacked plant protein and that of the same source converted to microbial protein. However, the additional nitrogen converted from ammonia to microbial protein may be determined on the basis of 100 gm. original straw. The organic nitrogen calculated on this basis is given in the third column of Tables V and VI. If then the original nitrogen content of the straw (0·307 per cent.) be subtracted, the difference represents the inorganic nitrogen immobilised during the decomposition of 100 gm. of material. This is generally referred to as the "nitrogen factor" of the material and varies up to about 1·2 according to the nature of the plant tissue. It may be viewed as a measure of the amount of microbial tissue which will develop on 100 gm. of any particular material. The "nitrogen factor" for straws and readily decomposable material usually lies between 0·7 and 0·9.

Table V. *Nitrogen content of straws rotted by pure cultures of fungi, 48 days at 30° C.*

Organism	Organic nitrogen	Loss of dry matter	Organic N on 100 gm. original straw	Nitrogen factor	Nitrogen equivalent
A. versicolor	1·34	29·29	0·95	0·65	2·22
A. flavipes	1·39	28·94	0·99	0·69	2·38
Trichoderma sp.	1·42	27·10	1·04	0·73	2·69
Phoma sp.	1·07	25·54	0·80	0·50	1·96
A. terreus	1·51	24·87	1·14	0·83	3·33
A. nidulans	1·23	20·29	0·98	0·67	3·30
A. fumigatus	1·35	16·67	1·13	0·82	6·14
Actinomyces sp.	1·55	13·46	1·34	1·03	9·95
A. niger	1·30	13·34	1·13	0·82	6·14

Discussion of results. The "nitrogen factors" of the oat straw rotting with pure cultures are shown in Table V. The differences observed are considerable, ranging from 0·50 in the case of *Phoma* sp. to 1·03 for

Actinomyces sp. This latter is unusually high and exhibits clearly the physiological dissimilarity between the actinomycete tested and the fungi. There is clearly no correlation between the "nitrogen factor" and the loss of organic matter, nor with the loss of any particular constituent. The differences observed are essentially varietal and inherent to the organism on any particular medium.

Table VI. *Nitrogen content of straws rotted by mixed floras*, 48 *days at various temperatures.*

Temp. °C.	Organic nitrogen	Loss of dry matter	Organic N on 100 gm. original straw	Nitrogen factor	Nitrogen equivalent
20	2·09	38·85	1·26	0·96	2·47
30	2·37	51·00	1·15	0·85	1·69
35	2·48	50·56	1·22	0·92	1·80
50	2·28	42·14	1·17	0·87	2·07

The figures obtained for straws rotted by mixed floras at several temperatures were determined for purposes of comparison and are given in Table VI. The "nitrogen factor" in these cases is the resultant of the many organisms involved and, in the period for which the experiment was carried out, had probably not quite reached stability. The mixed flora figures seem to be rather higher than those for the fungi, which would suggest that the "nitrogen factor" of bacteria on straw is probably well over 0·8 and probably of the same order as that obtained for *Actinomyces* sp. There is little doubt that in decompositions with a mixed flora the nitrogen converted to microbial protein by one organism may subsequently be utilised by another, and that there is a sequence in types. The "nitrogen factor" of the straw at an early stage of decomposition is frequently higher than later when a condition of stability has been reached, and it is clear that ammonification of a portion of the synthesised protein does occur.

The "nitrogen factor" is not directly a measure of the efficiency of any organism or group of organisms, since it is determined without reference to the loss of organic matter. For example, in Table V it is seen that oat straw rotted with *Aspergillus terreus* has a factor of 0·83 and with *A. niger* 0·82, yet in the former case there was an apparent loss of organic matter of 25 per cent. and in the latter only 13 per cent. If, however, the quantity of organic matter removed be related with the nitrogen immobilised in a new expression, a factor may be obtained which, subject to certain allowances, is a direct indication of the efficiency of the organism in decomposition. The term "structural nitrogen equi-

valent" or "nitrogen equivalent" is suggested for this factor, which may be defined as the nitrogen immobilised in the course of the removal of 100 gm. of organic matter from any given material. If the organism is very efficient and can utilise a considerable quantity of organic matter its "nitrogen equivalent" will be low; if less efficient it will be higher since more microbial tissue must be synthesised to assimilate the same quantity of carbohydrate. In the last column of Table V is found the "nitrogen equivalent" of the several organisms. *Phoma* sp. is seen from this to be the most efficient and *Actinomyces* sp. the least, the various *Aspergilli* falling into line in parallel with the loss of organic matter.

In Table VI the relative efficiencies of the various mixed floras developing at 20° C., 30° C., 35° C. and 50° C. are contrasted. It will be seen that the flora at 30° C. is the most efficient and that at 20° C. the least. Attention is particularly drawn to the high efficiency of the organisms at 50° C., a result which would not have been anticipated. The "nitrogen equivalent" of the mixed flora at 30° C. may be compared with the individual organisms at that temperature, given in Table V. The co-operative effect of the organisms in a mixed flora may well be seen, since 1·7 gm. of nitrogen as microbial protein in a mixed flora utilise as much carbohydrate as 2·0 gm. of nitrogen in *Phoma* tissue or 2·2 gm. of nitrogen in *Trichoderma* tissue.

This factor is, unfortunately, not of absolute value, but only proximate, for its accuracy is vitiated in two ways. In the first place the figure for loss of organic matter during decomposition is invariably too low, as explained above, owing to the synthesis of microbial tissue, and this has the effect of increasing the "nitrogen equivalent" above its time value. In the second place it only takes into account microbial nitrogen derived from inorganic sources, and neglects that obtained from the plant proteins when available. It is probable that in most cases the effect of this is small, since in the presence of excess of inorganic nitrogen it is unlikely that the plant proteins would be utilised to any extent. If, however, a case occurred in which insufficient inorganic nitrogen was present and the plant proteins were attacked and converted to active microbial tissue, then the "nitrogen equivalent" would be affected and rendered too low.

In spite of these objections it is claimed that for comparative purposes the "nitrogen equivalent" is a useful factor for expressing the efficiency of any organism in decomposing a particular material. It will, however, vary from material to material according to the availability of the carbohydrate constituents to the flora present. Particularly is

the "nitrogen equivalent" of a flora likely to be useful when investigating the rate of immobilisation of nitrogen in a decomposition, since the efficiency of the organisms is found to vary, being low at first and rapidly increasing. The standard "nitrogen equivalent" likely to be reached by a mixed flora on available materials such as straws is about 1·5, on the assumption that a 50 per cent. decomposition will be effected, and that the "nitrogen factor" is 0·75. The relative efficiencies of the organisms on straw as estimated by the proposed "nitrogen equivalent" are approximately comparable amongst themselves but not with the mixed flora decompositions, in which the nitrogen immobilised by one organism is assimilated and utilised by another as the sequence of active organisms changes.

III. GENERAL DISCUSSION.

In this paper has been presented a detailed account of the action of some common soil fungi in pure culture on sterile straws. The organisms tested seem very similar in their activities and in their power of assimilating the various straw constituents, varying only in extent of decomposition effected. It is possible that the decompositions may have been in part arrested by an accumulation of intermediary or by-products, for, in all cases at the close of the period of decomposition there was remaining additional available nitrogen, and carbohydrate material. It may be suggested that the straw had undergone extensive modification during the somewhat drastic process of sterilisation adopted, and that the substrate was therefore in no way a natural one. That some slight change occurs cannot be denied, but the evidence obtained from the decomposition of sterilised straws re-inoculated with a mixed soil flora and compared with that of unsterilised straw, indicates that the change is not large. That which there is, is in the direction of making it slightly more available, and is probably mainly a mechanical one caused by the cracking of the lignified walls. It may also be suggested that a state in which there is abundant available nitrogen present is unnatural and this also must be admitted except in the case of farmyard-manure heaps where there is usually a marked excess.

Such single factor studies form an essential preliminary to the synthetic investigation of mixed flora decompositions, by which method it is possible to trace the effect of organisms working in association. The part played by any organism in a mixed flora may be quite different in degree, though probably not vastly different in type, from that effected in pure culture. Its action may be retarded by the competitive action

of other organisms utilising the same constituents, by the production of unfavourable by-products, or by shortage of nitrogen for metabolic purposes. Its action may be accelerated or extended by the removal of its own intermediary products, or the raising of the temperature of the medium through thermogenesis. Without some differential method of attack it is impossible to assess the true rôle of any organism. It is proposed, therefore, next to carry out investigations in which these effects of association of organisms will be studied. Decomposition will be effected by one or more fungi in the presence of particular bacteria, and by this means and in several stages building up a synthetic flora.

IV. Summary.

1. A number of common soil fungi isolated from rotting straw were inoculated into sterile straw with the addition of available nitrogen.

2. The amount of decomposition effected, the losses of various straw constituents, and the immobilisation of nitrogen which took place, were all determined, and compared with mixed flora decompositions at various temperatures.

3. Though none of the organisms develop appreciably on a cellulose-agar plate, all were found to decompose cellulose to a considerable extent.

4. Certain minor differences in ability to utilise the carbohydrate constituents were noted, but in general all substances but lignin were attacked to a degree relatively proportional to the apparent total loss of organic matter.

5. The "nitrogen factor" or nitrogen immobilised by 100 gm. straw rotting with a particular organism was determined in each case. There is no correlation between the "nitrogen factor" and the loss of organic matter or of any particular straw constituent. The differences appear to be varietal.

6. Some indication of the relative efficiencies of the organisms was obtained from a factor for which the name "nitrogen equivalent" is proposed. This represents the nitrogen immobilised as microbial protein in the course of the removal of 100 gm. organic matter from any given plant material.

7. Attention is drawn to difficulties which restrict the absolute value of the "nitrogen equivalent" as a measure of efficiency in decomposition.

8. The value of single factor studies as a preliminary to the synthetic method of investigation of such a process as biological decomposition is discussed.

The author is indebted to Sir John Russell, F.R.S., Director of the Rothamsted Experimental Station, for placing at his disposal the facilities of that Station, and to the Department of Scientific and Industrial Research for a Senior Research Award, during the tenure of which this work has been carried out.

Especially are his thanks due to Dr W. B. Brierley, and Mr E. H. Richards, Heads of the Departments of Mycology and Fermentation, for their ready assistance and advice.

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REVIEWS

The Penicillia. By Charles Thom, with assistance of Dr Margaret B. Charch in handling the cultures and in the Medical chapter, Dr O. E. May upon the Biochemical chapter, and Dr M. A. Raines in preparing the illustrations. London: Baillière, Tindall and Cox, 1930. Pp. xiii + 644. 99 figs. Price 45*s*. net.

There are certain genera of fungi which most mycologists unashamedly avoid and which, if actually met with in the course of work, are regarded with almost comical dismay. From time to time mycologists of unusual courage attack such forms and produce assortments which may or may not introduce some degree of clarity: Dr Thom must undoubtedly take a leading rank among these heroes of science. By his volume on the *Aspergilli* he laid all mycologists in his debt and he has now consolidated his position by this monumental tome on the *Penicillia.*

The place of the *Penicillia* in our daily life can best be described in the author's own words: "They rot our fruit, attack our vegetables and meat, injure our stored grain, spoil our soft drinks and our bottled water, contaminate our pantries and kitchens, and even attack our bodies. They infect and at times destroy the usefulness of solutions and moist precipitates, discolour fibres, wood, paper stock, stored paper and sometimes our books. In the laboratory they infest and often invalidate every kind of culture operation, bacteriological, mycological, or phanerogamic. To offset these activities the chemists have gathered a little return by using them in biochemical investigations and the cheese industry has capitalised their enzymic activity to ripen such cheeses as Camembert and Roquefort. Otherwise their possibilities of usefulness remain mostly unknown, but their presence is thrust upon us so frequently that some means of identifying them is very desirable."

The author's study of these noisome weeds began in 1904, and its development is again best described in his own words. "In his first scheme of study [Thom, 1905] he attempted to follow the lines set by bacteriology in proposing to separate species upon the presence, absence, or intensity of selected biochemical reactions. This proved very useful in helping to fix upon fairly broad groups, but, within methods at that time deemed practical, it failed to furnish a satisfactory separation within related series. Carried to a quantitative chemical basis, some such scheme remains the only hope of fixing the individual strain, but this service can only begin when the resources of the morphologist in the culture room have determined the group involved."

"Even in the publication of his *Cultural Studies* (1910), he retained the futile hope of a cultural and morphological species-diagnosis which would make possible the identification of every *Penicillium.* By 1914, this was abandoned for the delimitation of groups presenting common morphological and cultural characters. In these groups, forms obviously morphologically related are brought together and the contrasting characters are mainly quantitative: the same structures a little larger or a little smaller. The same reactions, partially suppressed or variously accentuated, often give marked contrasts making for individuality in the strain or species, but frequently, in a series of cultures, merge so completely that the task of diagnostic description becomes endless. This was further developed in the study of the *Aspergilli* (1926) and is followed here."..."To lay a foundation for a permanent knowledge of this lot of moulds, the whole range of morphology and physiology must be searched for marks of separation stable enough, and sharply enough marked to convey to the reader a definite picture of the organisms studied. Then organism by organism they must be fitted into the scheme of classification to form a consistent and interpretable whole. That ideal has not been reached, but we hope that a tangible outline has been made and enough types established to insure group recognition at least for the more abundant moulds of the penicillate series."

The first quarter of the book concerns itself with general and historical introductory matter, herbaria and culture collections, generic considerations and usages, various methods of culture, observation and description, physiological and biochemical activities, and the distribution and significance of *Penicillia* in nature, industry and animal and human disease. These chapters are exceedingly useful summaries, full of valuable suggestion and practical wisdom, and one could wish that Dr Thom had written more fully on some of the issues he merely points to and then passes by. They are chapters that should be read by all mycologists and plant pathologists whether interested specifically in the genus *Penicillium* or not.

The remainder of the volume, some 450 pages, is devoted to a systematic survey of the forms accepted by the author as *Penicillia*, *i.e.* the genera *Penicillium, Gliocladium, Scopulariopsis* and *Paecilomyces*. The genus *Penicillium* itself is characterised into four divisions which are again divided into sections and sub-sections. The treatment is the same throughout; first a group diagnosis, then a key to the species followed by a detailed description of each species. The latter are numbered in order and the total number of species in the genus *Penicillium* is 443. Including the three other genera the number reaches 543. In chapter xxv undeterminable *Penicillia* are considered, a further 69 forms being described and in the following chapter 48 species described as *Penicillium* by previous authors are excluded from the genus. The volume closes with a chapter of group and special keys, 24 pages of bibliography, general and species indices and a short list of 24 species or varieties discussed as new or under new names. There are 99 illustrations.

Chapter xxvii commences "What then is a species?" and, as this is rather characteristic of Dr Thom's book, it will easily be understood that to discuss, even in a summary way, the points of interest and controversy he raises would lead to a very considerable essay. One cannot do this here, but one may express the opinion that this volume is destined to become one of the classics of botany and will long serve as a model for the treatment of other genera of fungi.

WILLIAM B. BRIERLEY.

Bacteriological Technique. By J. W. H. Eyre. London: Baillière, Tindall and Cox, 1930. Third edition. Pp. xii + 619. 238 figs. 21*s.* net.

The second edition of this work was published seventeen years ago, but the present edition remains little changed and cannot be said to reflect the advances made in practice during this period. Many new techniques derive from laboratories of agricultural bacteriology and plant pathology, but with a few exceptions the work of such research centres seems to be almost unknown to the medical bacteriologist; one can only think to his considerable loss. When compared with the old edition most of the old methods are still found, although a certain number of new ones have been added. Most of the revision is concerned with the *p*H adjustment of nutrient media, bacteriological analysis and methods of animal experimentation, and there is a new chapter on "Methods of testing pathogenesis and establishing active immunity."

Everyone will find sins of omission and commission in this volume, for technique is very essentially a personal acquisition. One can, for example, find no description of methods of examining faeces or urine, of preparing vaccines or even of performing a microscopical count of bacteria, the bacteriophage receives two pages and filterable viruses at large do not seem to be mentioned: on the other hand, the author's treatment of the fungi is a veritable sin of commission.

The volume is written essentially from the point of view of medicine, public health and animal experimentation, plant bacteriology being entirely neglected. It contains a good deal of practical information and will be a useful compendium to have available in the laboratory.

WILLIAM B. BRIERLEY.

Dictionary of Biological Equivalents, German-English. By E. ARTSCHWAGER. London: Baillière, Tindall and Cox, 1930. Pp. 239. 6 plates. 20*s.* net.

This is a useful volume to have on a convenient bookshelf. So far as can be seen, looking at random through the contents, the translations appear to be adequate and accurate and no misprints have been noted. Everyone will sympathise with the author's difficulty in selecting words to be included and to be omitted, particularly when space and price are limited, but plant physiology and microbiology do not appear to have received their due share of recognition. There are three pages of plant illustrations dealing entirely with plant phanerogams and three pages of animal figures illustrating birds, worms and arthropods: it is not easy to understand the choice of these particular figures. In addition there are units of weights and measures, lists of strong verbs, and frequently used German abbreviations. The price of the book would seem rather high, which is a very serious matter nowadays.

WILLIAM B. BRIERLEY.

An Index to the Chemical Action of Micro-organisms on the Non-nitrogenous Organic Compounds. By E. I. FULMER and C. H. WERKMAN, assisted by A. WIEBEN and C. R. BREDEN. London: Baillière, Tindall and Cox, 1930. Pp. xii + 198. 20*s.* net.

This is a summary of literature dealing with the action of bacteria, yeasts and moulds on non-nitrogenous compounds: only those instances are cited in which a named organism acted on a named substrate to produce a named compound. The survey is not intended to be complete and its purpose "is to cite references involving a variety of organisms, substrates, products, and authorities; that is, to give a cross-section of the field complete enough to introduce the reader to the subject." Only typical references are given to the more common types of fermentation, whilst for the more uncommon types the citation is fuller. Much of the older work has been omitted and there are few references prior to 1900. The contents of the book are in the form of three clear and conveniently arranged tables. The first is an alphabetical index to micro-organisms and substrates with their corresponding products and authorities in a second column. In Table II the substrates are arranged alphabetically as against their micro-organisms and authorities, whilst, in Table III, the products are arranged alphabetically. Thus the first table enables one to compare the chemical activities of different organisms; Table II gives an idea of the products elaborated from a given substrate by any particular organism and Table III enables one to find the types of substrate from which a given chemical is produced by a particular organism. There is a bibliography of 491 references. The book will be of use to all workers whose studies carry them into the rapidly developing field of zymology, but the price seems distinctly excessive and, as the book is essentially one for copious annotation and therefore personal possession, it is to be hoped that a second edition, if called for, will be produced more cheaply.

WILLIAM B. BRIERLEY.

Recent Advances in Entomology. By A. D. IMMS. London: J. A. Churchill, 1930. Pp. 374. 84 text-figures. Price 12*s.* 6*d.*

Within recent years few subjects in the field of biological science have made such strides as entomology, but with few exceptions this progress has not been due to the

encouragement given to its study by the universities or by other institutions interested in the search for knowledge for its own sake. On the contrary, the stimulus to entomological research has had its origin chiefly in connection with the more practical needs of mankind, such as the production of food and raw materials and the cure of disease, both human and animal. The need for a solution of problems of a purely economic character has led back to the investigation of the more fundamental questions of entomology, thus reversing the more usual course of events in which work in pure science has preceded the application of that science to some field of human endeavour.

Perhaps for this reason, students of entomology—and specially economic entomology—have tended to acquire a highly specialised knowledge in those directions more immediately concerned with their own particular work and to neglect advances made in other lines, while problems in urgent need of attention have been overlooked owing to the absence of any wide review of the field as a whole. Under these circumstances, Dr Imms's latest book should receive a very warm welcome, for under the title of "Recent Advances in Entomology" he has given a remarkably concise yet comprehensive survey of many of the problems with which modern entomology is concerned. The author states in his preface that his object has been "to review and discuss certain aspects of the subject along which recent advances have been fertile in new facts and ideas" and he expressly disclaims any attempt to make the work comprehensive. In point of fact, however, it probably approaches more nearly to the comprehensive than its author supposes, and while it would be ungrateful to criticise a book for not achieving something which it was never intended to do, it seems nevertheless permissible to urge that in a revised edition, or even by means of a second volume, certain further aspects of entomology should be discussed in the same admirable manner. For instance, insect nutrition and insect respiration are not dealt with, doubtless because these subjects either have recently been, or will shortly be, treated by others, but we feel that Dr Imms should not in future give too much weight to such considerations, because the value of his surveys is greatly accentuated by the fact that they are drawn from the wide point of view whereas the specialist may fail to see "the wood for the trees."

These remarks, however, must not be taken as suggesting that the contents of the present book are in any way scanty, as the following summary will show: Morphology receives 46 pages, Metamorphosis 14 pages, Palaeontology 29 pages, Sensory Organs (treated both from the morphological and functional points of view) 56 pages, Coloration 31 pages, Ecology 90 pages, Parasitism and Biological Control 96 pages.

Within the space of a review it is impossible to discuss the different subjects falling within so wide a field, but it may be mentioned that it is refreshing to find the coloration problem treated from the optical and biochemical aspects rather than from the evolutionary. It may also be pointed out that the ecological chapters are very comprehensive and range from the effects of changes of temperature upon insects to the varietal resistance of plants and the locust problem. In this connection, it may be questioned whether the term Ecology is not given too wide a significance in modern entomology. Admittedly fashion enters into these questions of terminology, but there seems little point in using the word for much that is quite adequately covered by the term Physiology, and which by analogy with vertebrate physiology or plant physiology can hardly be considered to form a distinct branch. However this may be, the reader will possibly find more than he expected in the chapters on ecology.

The last section of the book, on Biological Control, will be found both useful and timely as presenting a plain unvarnished account of a subject which has suffered more at the hands of its friends than of its enemies. While those actually concerned in the work of biological control have seldom made exaggerated claims in regard to its practical value, others less closely in touch have too often allowed their enthusiasm to outrun their discretion, and it is hoped that these chapters will be widely read by entomologists who have no experience of controlling insects in the field and who are apt to declare that the only scientific method of checking insect pests is by the so-called method of biological control. As the author shows, the results achieved under suitable conditions are so eminently successful that it would be regrettable if the

method were to be discredited by misguided attempts to apply it where conditions are adverse.

In conclusion, it may be mentioned that the book has good indices, both subject and author, and that considerable care has apparently been exercised in the references to literature so that while the important papers are mentioned the reader is not overwhelmed as is too often the case. A few misprints have been noticed (*e.g. Silicrura* for *Cilicrura* on p. 139, and *Teracolous* for *Teracolus* on p. 169) but they are not material, and Dr Imms is to be congratulated on producing a very useful book.

J. C. F. FRYER.

The Physiology and Biochemistry of Bacteria. By R. E. BUCHANAN and E. I. FULMER. London: Baillière, Tindall and Cox, 1930. Vol. II. Pp. xvi + 709. 57 figs. 34*s*. Vol. III. Pp. xv + 575. 2 figs. 34*s*.

The first volume of this massive work received notice in the *Annals of Applied Biology*, XVI, 2, 1929, and the final two volumes are now before us. Vol. I dealt with growth phases; composition and biophysical chemistry of bacteria and their environment; and energetics. Vol. II deals with the effects of environment upon micro-organisms and Vol. III with the effects of micro-organisms upon environment. It may be inherent in any treatment of such subject material that these two volumes are very much less readable than Vol. I, more a straight compilation of data rather than a narrative account. Most of the data are those of biochemical bacteriology, and in this huge field the work is so scattered and the data so essentially unrelated that a narrative treatment or any real synthesis would seem, at present, to be impossible. What the authors have done, and done with a thoroughness which is beyond praise, is to collect and systematise these data in an easily available form and, in thus setting in order the almost Augean stables of the subject, they have performed a Herculean task. Whatever sins of omission or commission may be found in these volumes, they stand out as a landmark in the history of bacteriological writings.

Vol. II is divided into three sections: Section A, headed "Effects of environment. Recognition and measurement," and containing but one chapter in which are discussed rates of reaction and problems of determination and comparison: Section B, headed "Effects of physical environment upon micro-organisms," and containing three chapters dealing respectively with effects of temperature, effects of rays and of emanations, and effects of various physical environments upon micro-organisms: and Section C, headed "Effects of chemical environment upon micro-organisms," and containing four chapters dealing first in general with the effects of the chemical environment and then with the effects of inorganic compounds and their ions, effects of non-nitrogenous organic compounds and finally the effects of the nitrogenous organic compounds. Vol. III has only two sections, Section D, headed "Special physiological inter-relationships of micro-organisms," containing one chapter on commensalism and symbiosis, and Section E, headed "Effects of micro-organisms upon their chemical environment," and containing five chapters dealing respectively with enzymes and catalysts; general consideration on chemical changes produced by micro-organisms; changes produced in inorganic compounds; changes in non-nitrogenous carbon compounds; and finally, changes in nitrogenous organic compounds.

The above brief summary of the contents will give some idea of the range of territory covered, and the devotion of the authors may be appreciated when it is realised that in Vol. II the literature cited contains nearly 2300 references and in Vol. III nearly 2700 and that European literature is conspicuous by its abundant presence. In addition to the bibliography there are at the end of each volume an author index, a subject index and an index to micro-organisms.

The literature survey is, of course, very far from complete, but to attempt a complete survey would be almost impossible within any reasonable limitations of size,

and, in any case, such monographic treatment is far better done by individual specialists for their own particular fields of work.

The authors describe their work as a treatise "intended to serve merely as an introduction to the subject," but, if three massive volumes containing 1800 at times unreadably packed and technical pages constitute merely an introduction, one cannot help wondering what would be the authors' conception of, shall one say, a moderately full treatment of the subject. The authors are really too modest, for in their treatise, which is characterised by a wide sweep and massive coherence of treatment, they have produced a book which immediately takes rank among the major works of modern biological science and one which is likely to serve as the standard work on the subject for many years to come.

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REPORT OF THE COUNCIL OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS FOR THE YEAR 1930

During 1930 the Association has met on seven occasions. At five of these, various subjects of interest were brought before the Association by members and visitors, to whom the Association is greatly indebted.

An outstanding event of the meetings was Dr Butler's Presidential Address on: "Some Aspects of the Morbid Anatomy of Plants."

On May 16th, the Association held a Field Meeting at the Forest Products Research Laboratory, Princes Risborough, and members and visitors were entertained to tea by the Director, Mr R. S. Pearson, to whom we are indebted for one of the most successful Field Meetings we have had recently.

An invitation from Professor Stenhouse Williams to visit the National Dairying Research Institute at Reading was accepted, but unfortunately the meeting had to be cancelled because of unforeseen difficulties in the arrangement of dates.

The attendance at meetings has varied from 25 to 80, the average being 48; the proportion of members to visitors has been about equal.

During the year, the Association has lost four members through resignation, and the Council have, with regret, to record the death of Dr James Waterston, a former Secretary and Vice-President of the Association, Mr F. V. Theobald, one of the original members, and a former president, Mr C. C. Brooks and Mr M. H. Sutton.

The number of new members elected during the year was 16, which gives an increase in membership of 12. The Association now numbers 289 ordinary members and 12 honorary members.

The Royal Society invited the Association to nominate two members to represent it on the International Union of Biological Science, and the Council invited Dr Butler and Dr Imms to represent them.

Dr Butler having been nominated for one year only, retired in June last, and the Council invited Dr W. B. Brierley to serve in his place.

During the past year the Association has again enjoyed the privilege of holding its meetings in the Botanical Department of the Imperial College of Science and Technology, and the Council feel sure that the Association will wish to take this opportunity of recording its grateful thanks to the college authorities for this valued hospitality.

Papers read to the Association during the year 1930:

Jan. 24th. Presidential Address by Dr E. J. Butler, F.R.S.: "Some Aspects of the Morbid Anatomy of Plants."

Feb. 21st. Dr W. R. Thompson: "Biological Control of Injurious Insects and Weeds."

March 21st. Messrs R. G. Hatton, T. Wallis, Dr Swarbrick and Mr N. H. Grubb: "The Nutrition of Fruit Trees."

Oct. 24th. Mr M. A. Tincker, Mr M. G. Jones, and Dr P. S. Hudson: "Factors influencing Yield of Certain Cereal Crops."

Nov. 21st. Dr H. F. Barnes: "Specific Resistance of Willows to Insect Attack."

Dec. 12th. Messrs D. Ward Cutler and E. H. Richards: "Purification of Waste Waters from Sugar-Beet Factories."

REPORT OF THE HONORARY TREASURER FOR THE YEAR 1930

During the year ending December 31st, 1930, subscriptions and entrance fees from members amounting to £346. 7*s*. 8*d*. were received. This sum represents a reduction of £9. 5*s*. 4*d*. as compared with the previous year. An actual increase would have presented itself were it not for the fact that members' subscriptions, two years or less in arrears, and amounting to £51. 5*s*. 0*d*. were still owing. It is requested that all members who are still debtors to the Association will discharge their obligations as promptly as possible in order that these arrears may be cleared.

The working expenses of the Association, apart from the actual cost of *The Annals of Applied Biology*, amounted to £23. 7*s*. 0*d*. which is a reduction of £9. 13*s*. 0*d*. as compared with the previous year. The publication account with respect to Vol. XVII of the *Annals* showed an increased balance against the Association amounting to £215. 18*s*. 11*d*., more than for Vol. XVI. The total sum due to the publishers was £510. 17*s*. 11*d*., an amount that is considerably in excess of that of previous years. This increase is due almost entirely to the markedly augmented size of Vol. XVII and to the fact that 600 copies are now printed as compared with 500 formerly issued. The year closed with a debit of £125. 10*s*. 8*d*. in expenditure over income for that period. After all obligations had been met the assets of the Association amounted to £744. 18*s*. 6*d*. of which £581. 5*s*. 0*d*. was represented by National Savings Certificates.

A. D. IMMS,
Hon. Treasurer.

THE ASSOCIATION OF ECONOMIC BIOLOGISTS

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED DECEMBER 31ST, 1930

Dr

EXPENDITURE

	£ s. d.	£ s. d.
To *Annals of Applied Biology*:		
Estimated Value of Stock at January 1st, 1930	60 3 2	
Expenditure during 1930	510 17 11	
	571 1 1	
Less Estimated Value of Stock at December 31st, 1930	48 13 1	
		522 8 0
To Printing and Stationery		10 16 0
Addressograph Machine and Accessories		7 0 1
Postages and Cheque Stamps		6 19 4
Sundry Out-of-Pocket Expenses of Secretaries and Treasurer		5 3 7
Audit Fee Reserve		4 4 0
		£556 11 0

Cr

INCOME

	£ s. d.	£ s. d.
By Subscriptions:		
Arrears	11 9 0	
Entrance Fees	8 18 0	
Current	326 0 8	
		346 7 8
By Contributions to cost of papers etc. in *Annals of Applied Biology*		55 0 0
By Interest on National Savings Certificates and Bank Deposit		29 12 8
By Balance, being excess of Expenditure over Income for the year		125 10 8
		£556 11 0

BALANCE SHEET, DECEMBER 31ST, 1930

LIABILITIES AND SURPLUS

	£ s. d.	£ s. d.
Sundry Creditors:		
Cambridge University Press	510 17 11	
Addressograph Ltd.	7 0 1	
Audit Fee Reserve	4 4 0	
		522 2 0
Subscriptions paid in advance		11 14 0
Excess of Assets over Liabilities:		
As Balance Sheet of December 31st, 1929	870 9 2	
Less Balance of Income and Expenditure Account for 1930	125 10 8	
		744 18 6
		£1278 14 6

ASSETS

	£ s. d.	£ s. d.
Cash:		
At Bank on Current Account	247 11 5	
At Bank on Deposit Account	350 0 0	
		597 11 5
Debtors for Subscriptions 2 years or less in arrear and considered good		51 5 0
500 National Savings Certificates		581 5 0
Stock of *Annals of Applied Biology*, at estimated value		48 13 1
		£1278 14 6

A. D. IMMS, *Honorary Treasurer.*

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association.

H. J. COX & CO. *Incorporated Accountants.* } *Auditors.*

HARPENDEN, *February 6th*, 1931.

Vol. XVIII, No. 3 August, 1931

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF ECONOMIC BIOLOGISTS

BY

W. B. BRIERLEY

AND

D. WARD CUTLER

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VOLUME XVIII AUGUST, 1931 No. 3

THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS

II. FURTHER STUDIES ON THE MOVEMENT OF MOSAIC IN THE TOMATO PLANT[1]

BY JOHN CALDWELL, B.Sc., Ph.D.

(*Department of Mycology, Rothamsted Experimental Station, Harpenden.*)

(With Plates XVII–XX and 1 Text-figure.)

CONTENTS.

INTRODUCTION.

IN the first paper of this series, the results of some experiments on the movement of the agent of the virus disease—aucuba mosaic of tomato—in tomato plants were described and discussed. It was shown that the agent tended to move freely through the plant, that the movement was apparently not restricted to the vascular tissue and that no movement took place through dead tissue or, normally, in the water stream in the xylem vessels. When a portion of the stem was steamed so as to destroy all the living tissues in the treated area the virus was found to be unable to cross that region and to infect the upper part of the plant if it had been inoculated into the lower, and conversely. When Chinese ink was introduced into the xylem vessels in the lower part of the stem, particles were

[1] A grant in aid of publication has been received for this paper.

found to pass into the normal upper part of the plant, across the steamed area. This demonstrated that protein plugs had not been laid down in the vessels; such plugs might conceivably have filtered the virus out of the upgoing water stream. The data obtained suggested that the virus agent of aucuba disease of tomato did not travel, as do the normal metabolites, chiefly in the vascular tissues proper, that movement could take place freely through any living tissue, there being no localisation of the movement as was found in the case of dyes, metabolites, etc., and that movement through non-living elements was normally not possible. This line of investigation has been continued, and that paper deals with the results of these subsequent groups of experiments.

EXPERIMENTS WITH TOBACCO MOSAIC IN TOBACCO AND IN TOMATO.

The experiments with aucuba disease of tomato were repeated with tobacco mosaic in tobacco and in tomato. The variety of tobacco used was *N. tabacum* var. White Burley, that of tomato *S. lycopersicum* var. Kondine Red. The aucuba mosaic material was the standard material of this station, originally obtained from Dr Bewley at Cheshunt. The tobacco mosaic had been obtained indirectly from Dr Johnson and was his No. 1 strain. The same procedure was adopted in these experiments as in the previous ones. An internode of a tomato stem was steamed in the manner described in the earlier paper, so that all the living cells were killed without interfering to any great extent with the water stream in the xylem vessels. Thereafter, inoculations were made from macerated tissue of leaves from a tomato plant infected with tobacco mosaic. In this case, as before, when the inoculation was made in the lower part of the plant, the symptoms appeared in that part: when the inoculation had been made above, only the upper part of the plant was affected. There were no cases in which infection had spread across the lesion. Even when the upper uninfected part of a plant inoculated below the lesion was removed, planted as a cutting, and allowed to grow for some weeks, on no occasion did symptoms appear in that portion. The top of the plant was removed above the lesion by cutting with a red-hot scalpel. This had the effect of searing the cut end and precluded the possibility of accidental infection of the living cells. The tomato cuttings "strike" very easily since the root initials are well formed before the stem is cut. The appearance of a stem with a cluster of roots above the steamed portion is shown in Plate XIX, fig. 3.

In the experiments with tobacco plants, even more care was required than in those with tomatoes. The internodes of tobacco plants at the

stage most suitable for these experiments are rather short. Further, there is no great development of mechanical tissue in the stem of this plant. As a result of these two factors, when the stems had been steamed, the leaves of the upper portion tended to fall over on to the lower. In tomatoes, on the other hand, the stems of plants $1\frac{1}{2}$–2 feet high are sufficiently well provided with mechanical tissue to permit of their remaining rigid after treatment with only slight support. It is the more important to prevent the upper and lower leaves of tobacco from rubbing together in these experiments, since the thick mat of glandular hairs which invests the upper and lower sides tends to make the separation of two leaves lying together a rather delicate task if the hairs must not be broken. It should be borne in mind that aucuba mosaic of tomato and simple tobacco mosaic, both in tobacco and in tomato, are so contagious that damage to the trichomes under non-sterile conditions may lead to the infection of the plant. To prevent any difficulty arising from these considerations the upper part of the plants were enclosed in paper collars to prevent the drooping of the upper leaves on to the lower. When this was done it was found that the upper leaves remained fresh for a period of a fortnight and that, thereafter, the upper portion of the plant might be planted as a cutting. With these plants, again, it was found that no movement of the virus agent took place across the lesion. This fact brings this virus, which is closely similar in other respects to that of aucuba mosaic, into alignment with it as regards the methods of translocation in the plant.

EXPERIMENTS ON THE ABSORPTION OF VIRUS JUICE THROUGH A CUT PETIOLE.

It has been shown that solutions may be sucked backwards into the stem of a plant through the cut end of a petiole. It seemed reasonable to suppose that virus juice might equally well be used in this type of experiment and that the juice would pass directly into the xylem vessels of the main stem. The juice used in these experiments was filtered under pressure through macerated filter paper, or through a filter paper impregnated with fuller's earth, which removed all the solid material present in the macerated tissue. It was found that of this juice 1–4 c.c. were absorbed at the cut end of the petiole in 24 hours. The lamina of a leaf was removed under water and the cut end of the petiolar stump was inserted immediately thereafter into a small tube full of the extracted juice.

A large number of plants have been so treated, about eighty in all. It has been found that in the majority of cases where the petiolar stump

had been left attached to the plant after treatment the plant showed symptoms of disease in the usual manner after a period of incubation. It is not surprising that in these cases all the plants did not take virus disease. To ensure that the vessels would be of large size well-grown plants were taken, for example, about 1½ feet in height, and at this stage only under very exceptional conditions do more than a small majority of the plants develop symptoms even after inoculation by leaf mutilation. When young plants were used, though the amount of inoculum absorbed was very much less than that absorbed by the older plants, the incidence of infection was much higher, often 100 per cent., which is found also with leaf mutilation. The amount of living tissue exposed at the end of a cut petiole is naturally very small and, as has been seen, the virus agent travels only in the living tissues. When the petiole, following treatment as described, was removed from the plant with a sterilised scalpel after a period of less than 48 hours, it was found that the plant did not develop any symptoms of disease. In every case the petiole was removed close to the stem with a red-hot scalpel—being rather burnt than cut off. As a consequence, all the living tissues near the point of cutting were completely destroyed. This had the effect of preventing any chance infection arising from the liberation of infectious juice from the xylem vessels and the accidental inoculation of the living tissues around. Only four out of the sixty plants so treated ultimately developed symptoms of aucuba mosaic, and these symptoms appeared so long after treatment as to suggest the possibility of secondary, accidental infection. The results of some of the experiments are summarised in the appended table.

Table I.

Experiments on petiolar absorption of virus juice.

Date of experiment	Petiole removed within 48 hours		Petiole left attached	
	No. of plants	No. infected	No. of plants	No. infected
26. v. 30	4	0	4	0
14. vi. 30	—	—	8	7
20. vi. 30	—	—	8	6
14. vii. 30	6	0	6	2
18. vii. 30	3	0	3	2
13. viii. 30	6	0	6	5
Totals	19	0	35	22

It was concluded from these experiments that the virus agent was travelling with the juice in the xylem vessels of the main stem, but that there was no mechanism whereby it could get out of those vessels to infect the living cells of the mesophyll, etc. In the former instances, where the petiole had been left attached to the plant for some time after

treatment, the virus agent had had time to infect the exposed living tissue, to multiply in it and to travel through the protoplasmic connections into the cells of the main stem and thence into the plant as a whole. Apparently, the time taken by the agent to enter the broken cells at the end of the petiole, to infect the living tissue round it and pass into the main stem is considerable. Part of the time must necessarily be taken up in the multiplication of the virus, but a proportion is taken up in the movement of the agent across the living tissues. The rate of this movement is demonstrably slow, and this, along with other facts, suggests that movement takes place along the protoplasmic strands rather than as a mass movement along the phloem elements.

In an attempt to follow the path of the virus juice in the plant after absorption had taken place at the cut end of a petiole methylene blue or eosin was mixed with the juice. The fact that the virus agent passes unaltered through fuller's earth and other negatively charged colloids suggests that it itself carries, or that it is associated with some substance carrying, a negative charge. This consideration, coupled with the fact that the walls of the xylem vessels are slightly negatively charged, suggested the use of eosin in experiments demonstrating the absorption of the virus juice. The main difficulty which arises in experiments with eosin is that the dye is toxic and, if in sufficient concentration to be easily seen in the xylem vessels of the plant, is strong enough to kill the tissues and, ultimately, the whole plant. To avoid this difficulty, methylene blue was used. This dye carries a positive charge and is, therefore, slightly adsorbed by the walls of the vessels. This is no argument against its use in this connection but rather a point in its favour, since, if it can pass over a given region despite adsorption, clearly a substance of comparable size which has not been adsorbed and which, therefore, normally would move more rapidly, should also have travelled. This dye was used in fairly high concentration also and it was found that those plants which absorbed through a cut petiole the methylene blue-virus juice solution did not subsequently develop disease symptoms. It was also found that when a mixture of methylene blue and juice was inoculated into the lamina of a leaf in the usual manner of inoculation by leaf mutilation, the plants did not develop virus symptoms. Symptoms developed however after inoculation with the same methylene blue-juice preparation when diluted and the agent did not appear to be inactivated, when, after treatment with strong methylene blue, the juice was passed through fuller's earth. This led to the detailed examination of the plants, the petioles of which had been treated with strong

methylene blue and juice, when it was found that the living cells at the end of the petiole and near the injected vessels, and also those in the mesophyll round the needle holes in the laminae which had been inoculated, had been killed as a result of the toxic effects of the methylene blue. In these plants, therefore, the probability is that the virus agent had not actually come into contact with the living cell and, therefore, the plants could not truly be said to have been inoculated with the virus agent.

The effect of crushing leaves after injection of the xylem elements with virus juice.

As has been seen, there is no evidence that the virus agent is able to enter the living cells of a plant if it be moving about in the xylem stream. As it was clear that the juice had entered the xylem vessels and was, presumably, being carried up in the water stream to the leaves, it appeared reasonable to expect that if, in the experiments where no disease symptoms had followed the removal of the treated petiole, the virus agent had been allowed access to the living tissue the plants would have become infected. When this type of experiment was carried out it was found that crushing of the leaves above the treated petiole did give rise, in some cases, to the appearance of symptoms.

The method adopted was as follows. The cut end of a petiole was inserted into filtered juice. After an interval of 24–48 hours the treated petiole was removed from the plant with a hot scalpel. The leaves directly above the treated petiole were crushed with sterilised forceps. Only those leaves were crushed into which it was presumed that the virus juice had passed. The passage of the eosin was taken as a criterion in this case. A solution of eosin in the infective juice was sucked up by a plant, and was used as a control. The appearance of such a plant is shown in Plate XVII. As a consequence of the crushing, the contents of the xylem vessels were brought into contact with the living cells of the mesophyll. In the first series of experiments all the plants treated (six) developed symptoms of disease after the usual inoculation period. In another, nine out of thirteen plants with the leaves crushed became diseased. The success of this method depends on the distribution of the agent in the plant and on its effectual entry into the protoplast of a mesophyll cell. The amount absorbed and, consequently, the amount which is carried along the xylem vessels is, obviously, dependent on the tension which exists in the xylem vessels. Therefore, while on some occasions the movement of juice into the plant was rapid and the amount absorbed considerable, on others the rate of ingress was slow and the

amount absorbed small. Again, there tends to be a precipitation of colloidal and other materials when the filtered juice stands overnight and, when this precipitate is heavy and the rate of inflow low, the xylem vessels at the cut end of the petiole tend to become blocked with detritus of one kind or another. In such cases, the amount of material passing into the xylem stream is small, the concentration of the agent tends to be very considerably reduced and its distribution in the plant rather limited. As a result of these factors, the non-success of some experiments is hardly a matter for surprise. Sometimes none of the plants treated developed symptoms at all. On the other hand, in no experiments did the controls, of which the leaves had not been crushed after the removal of the petiole, develop any symptoms of aucuba disease. Clearly the actual crushing of the leaves of tomato plants has caused the appearance of symptoms only when the virus agent has been present as a consequence of inoculation of some kind. The results of some of the experiments are summarised in Table II.

Table II.

Experiments on petiolar absorption, with removal of petiole and crushing of leaves.

Date of experiment	Plants treated	Infection after crushing leaves
28. vii. 30	6	6
13. viii. 30	6	5
15. ix. 30	13	9

EXPERIMENTS ON THE ABSORPTION OF VIRUS JUICE BY THE PETIOLES OF PLANTS WITH STEAMED STEMS.

It was thought desirable to combine the technique adopted in the experiments here reported with that adopted in the earlier experiments before discussed (2). The middle portion of the stem of some plants was killed with steam in the manner described. Thereafter, the distal portion of a petiole on the lower part of the plant was removed under water and the end of the stump immediately inserted into filtered virus juice. The plants were then divided into two groups. In the first group, the treated petiole was removed with a red-hot scalpel within 24 hours close to the stem; in the second, the petiole was left attached. The upper leaves of the plants of the first group were crushed with sterilised forceps, and, after the usual period of inoculation, symptoms appeared in the part of the plant above the lesion. The plants of the second group were set aside without further treatment, and, after an appropriate interval, symptoms

appeared on the lower part of the plant. In the first group, on the other hand, the lower part of the plant showed no symptoms. In this experiment, it was therefore clearly shown that it is possible to inject a vascular strand, or strands, with virus juice, and not have infection spreading into the plant as a whole, whereas, if infection takes place as a result of the liberation of the agent from the vessels and its entry into the living mesophyll cells, the agent travels freely about the living tissues of the plant but is unable to move across an area of dead cells and so infect the living cells on the other side.

The entry of the virus agent into the xylem vessels.

It is clear that where the virus agent had actually been introduced into the xylem vessels it had moved upward with the water stream into the upper part of the plant, even across the region of dead cells. This does not imply that under normal circumstances the agent could travel in the water stream. It has already been seen that there is apparently no movement of the agent out of the water column and it is, therefore, difficult to see by what mechanism it could normally enter the vessels. With a view to testing this point the following experiment was set up. A series of plants were treated as before described to destroy the living tissue of one of the internodes. The lower part of each plant was then inoculated with aucuba mosaic. The usual method of leaf inoculation was not very satisfactory for this type of experiment, as it is possible that some of the agent might accidentally be inoculated directly into the xylem vessels during leaf mutilation. For this reason, a method of inoculation by rubbing the surfaces of the leaves with wool soaked in virus juice was used. In this method the hairs on the surface of the leaf are broken off by friction, and inoculation is made into the exposed protoplasts at the base of each. This method is, probably, more effective as a method of inoculation than most for the reason that it occasions the minimal amount of damage to the tissues (see Holmes (5)). After an interval of 8 days, when the virus had spread freely through the lower part of the plant and symptoms had actually appeared on the leaves of the axillary buds, the upper leaves were crushed in the usual manner. After a further interval of 6 days the upper portion of the plant was removed and was planted as a cutting. In no case did any disease symptoms appear. This experiment has been repeated and in no case has any infection occurred. These experiments, taken in conjunction with the earlier series in which the tissues of the upper portion were found to be non-infectious on inoculation into healthy seedling plants (see Caldwell (2)), lead one to the

conclusion that in the normal plant the virus agent does not travel in the xylem vessels—that, in fact, the virus agent does not enter the xylem vessels, since no mechanism exists which would normally admit of its entry, and that, further, even if the virus agent had entered the xylem vessels and had travelled in the water stream, there exists no evidence that the infectious principle would be able to leave the vessels and to enter the living cells of the mesophyll to set up infection.

THE ABSENCE OF THE VIRUS AGENT FROM THE HYDATHODE EXUDATE OF DISEASED PLANTS.

The results which have been outlined and discussed in this paper have all tended to show that the virus agent does not travel in the xylem stream in the normal plants. Only when infectious juice had been introduced directly into the vessels was the agent found to be carried in the water stream. Even under these circumstances, the agent did not cause infection unless the contents of the vessels had been brought into close contact with the living mesophyll cells. For example, it was necessary to crush the leaves in some experiments before any symptoms appeared on the treated plants. It was concluded, therefore, that the agent of aucuba mosaic, at least, was unable to leave the xylem vessels, and it is suggested that a similar difficulty would arise in connection with its entry into the xylem stream. In other words, in the normal diseased plant there is no virus material in the water stream. This point can very easily be tested experimentally. The leaves of the tomato are furnished with hydathodes of a simple type. These function but rarely under ordinary glasshouse conditions. On the other hand, when young plants are put under a bell-jar in a warm glasshouse so that the air inside the bell-jar is rapidly saturated with water vapour, drops of water appear after some time at the tips and along the margins of the leaves. These drops are exuded from hydathodes which are of the type described by Haberlandt as "water stomata" (cf. Haberlandt (3)). In such a hydathode the pore is more or less directly connected with the bundle ends and no secretory process as such is involved in the exudation of water from the pore. Plate XIX, fig. 2, shows the appearance of the pore at the tip of a young tomato leaf. It can be seen from this photomicrograph that it has a similar structure to the water stoma of *Fuchsia* as illustrated by Haberlandt (*loc. cit.*) (cf. Fig. 198 of Haberlandt, *loc. cit.*).

There is, between the bundle end and the pore proper, a group of loosely packed mesophyll cells and no secretory cells such as are characteristic of the glands found on the leaf of *Lathraea* spp. The liquid which

appears is, therefore, approximately of the nature of that found in the xylem stream. It was found that the exudation of water occurred most readily on the younger leaves of the axillary shoots which developed on the stump of a fairly mature tomato plant. The top of a diseased tomato plant was removed, leaving a stump of stem 4–6 inches high with five leaves on it. These leaves were removed and shortly afterwards the axillary shoots appeared. The leaves on these shoots were all badly infected with aucuba mosaic. The plants were put under bell-jars standing in water. After a short time in a warm glasshouse, drops of water appeared at each of the hydathodes. These were collected and inoculated into healthy seedling tomatoes. All the seedlings grew and remained healthy. Three sets of six plants were used in this experiment. As a control, leaves were removed from the same experimental plants and the drops of water which collected at the cut end of the petiole were inoculated into young seedlings. These drops, it was suggested, were contaminated by traces of the contents of the cut cells at the ends of the petiole. All the seedlings inoculated with this water developed symptoms. It was concluded, therefore, that only a trace of virus, as has already been shown by various workers, was required to infect the seedlings, and that even this trace was absent from the water exuded from the hydathodes. The water in the vascular tissue was therefore apparently free from virus.

Experiments with cuttings which have been kept in virus juice.

A simple method of demonstrating the non-movement of the virus agent from the xylem stream has been tried. The upper 6–9 inches of the stem of young tomato plants was removed and the end put into filtered virus juice. After 24 hours the tops were divided into three groups. The first group was planted as cuttings directly. The second was planted after the lower 2 inches of stem had been cut off with a red-hot scalpel. The third group was treated as was the second, and after planting some of the leaves were crushed with a pair of sterilised forceps. All the plants of the first group developed symptoms of mosaic as a result of the treatment to which they had been subjected. None of the plants of the second developed symptoms, because, as has been shown, the agent had not had time to pass upwards through the living tissue before the end was removed. Of the third group four of the eight plants treated developed symptoms of mosaic.

A similar set of experiments was carried out to ascertain if the leaves of plants with virus juice in the xylem vessels afforded suitable

material for inoculation. Two groups of cuttings were used. In the first the ends of the cuttings were placed in filtered virus juice for 24 hours. In the second the middle portion of each of the stems was steamed before the ends were placed in similar juice for a like period. The upper leaves of both sets were taken, macerated in the minimum amount of water and inoculations made from them. The plants inoculated developed symptoms of mosaic after the usual period.

The results of these experiments again come into alignment with those of the earlier experiments and confirm the results obtained therefrom.

DISCUSSION.

These experiments raise some new points which might be discussed at this stage. It appears that the virus agent can enter only slightly injured cells and may be incapable of passing directly into uninjured ones. As has been seen (4), neither in the case of virus juice placed on unbroken hairs on the surface of a leaf, nor in the case of that present on the inner boundary of living cells, *i.e.* in the xylem stream, do symptoms appear in the plant.

In these experiments, in which concentrated methylene blue was inoculated with the infectious juice, the tissues round the site of inoculation were killed and, as has been noted, the plants did not subsequently show symptoms of disease. This is attributed to the fact that movement apparently does not take place across a region of dead tissue. This explanation accounts also for the fact recorded by Holmes (5), viz. that washing off the inoculum from infected *N. tabacum* tissue on leaves of *N. glutinosa* "never decreases the number of successful inoculations, and may increase the number, especially if the fluid sample containing the virus to be measured also contains some substances harmful to the tissues of the inoculated plant." These observations affect the interpretation of results where the precipitants used in the preparation of virus material are themselves toxic to living cells. One might have been inclined to the view, which apparently has no justification from the subsequent experiments, that methylene blue in high concentration had inactivated, perhaps by adsorption, the virus agent. This point is dealt with more fully in a subsequent section.

THE NON-MOVEMENT OF THE VIRUS AGENT THROUGH KILLED MESOPHYLL CELLS.

Mention has been made of the difficulty of successfully inoculating plants with virus agents in the presence of toxic substances in the inoculum. It is suggested that, even where the amount of toxin is not

sufficient to kill large portions of the lamina, too severe injury to the cells round the points of inoculation may successfully prevent entry of the virus agent into the general plant body. This point is illustrated by some of the experiments on the effect of precipitates on virus juice. Holmes (5) finds that the rubbing of the lamina to break the hairs is a more effective method of inoculation than leaf mutilation—a point which seems to support the view that badly injured cells do not allow of the multiplication of the virus agent. The rubbing method is by far the most efficient for use with aucuba mosaic—100 per cent. infection taking place even with fairly mature plants.

The whole question of the technique of inoculation seems to be involved in this consideration. Tobacco mosaic and aucuba mosaic of tomato are both so infectious that little difficulty attends their successful inoculation into healthy plants. Even with these, however, there is some evidence to show that the less injury done to the inoculated tissues the greater will be the chances of infection. It is possible, though so far there is no evidence on this point, that in some of those instances where successful insect infection is comparatively easy and successful needle inoculation rare or absent, the non-success of needle inoculation is to be accounted for on the grounds that too much damage is done to the surrounding tissues and that insect attack is more efficient and less destructive. The insects which do act as vectors are, typically, insects with efficient sucking apparatus and not biting insects which crush and destroy the tissues of a leaf.

Experiments on the removal of the agent from infective juice.

There seems little doubt that the agent of aucuba mosaic itself carries, or is associated with some substance carrying, a negative charge. In many experiments with fuller's earth, which has been found an excellent material for removing much of the colloid material from plant juices before inoculation, there has been no occasion on which the infectivity of the treated juice has been obviously impaired as a result of treatment. There does not appear to be any saturation of the charges in the fuller's earth by the virus agent, as is found in the case of acid aluminium hydroxide. In this latter case, small quantities of juice after passage through the hydroxide may no longer be infective (cf. Allard (1)). The amount of the reduction of the infectivity seems to be associated with the volume of the juice which passed over the hydroxide, the positive charges thereon being readily saturated so that the negatively charged agent is no longer adsorbed (cf. Rhoads (6)).

With these facts established, it appeared that positively charged dyes, such as methylene blue, would be valuable as indices of the amount of movement of the virus agent in the xylem vessels. The negatively charged colloids of the virus juice should, theoretically, travel more readily than the positively charged methylene blue. As has been seen, when strong methylene blue was used the juice absorbed by the petioles did not, even when the petioles were left on the stem, infect the whole plant with mosaic. This, it is suggested, was due to the toxic effect of strong methylene blue and not to the inactivation of the agent by adsorption (cf. Vinson and Petre (9)).

An attempt was made to discover if the usual protein precipitants removed the virus agent from infectious juice. An acid solution of mercuric sulphate (see West, Scharles and Peterson (10)) was first used. It was found that a few drops of this precipitant were sufficient completely to discharge all the precipitable materials from the juice. When this was done and the precipitate made up with water and inoculated into plants no symptoms appeared. Neither did they appear when the filtrate was used as the inoculum. When, however, in a second set of experiments, the precipitate and the filtrate were neutralised with dilute alkali before inoculation the precipitate was found to contain the virus agent apparently unaltered. The filtrate was non-infectious even after treatment with sodium sulphate and zinc to remove any traces of toxic mercuric salts. In the case of the precipitate, the toxic effect of the acid had been removed and the virus agent was able to enter the living protoplasts of the mesophyll cells round the points of inoculation.

Other precipitation methods were tried. The fact that the material has to be heated in the method involving the use of zinc hydroxide makes this method unsuitable for the testing of the effect of this precipitant on the agent. Fairly prolonged heating on a water bath at 100° C. will inevitably destroy the agent.

The lead acetate method, on the other hand, is suitable for this investigation, and it was found that, when sufficient lead acetate was added to precipitate completely the proteins of the juice of tomato leaves infected with aucuba mosaic, all the virus appeared in the precipitate: the filtrate contained no virus as judged by inoculation into healthy seedlings even after the removal of the excess lead acetate with sodium carbonate.

Experiments on the movement of the virus agent through living tissues.

The experiments recorded above, together with those described in the earlier paper, indicate that movement of the virus agent tends to be, normally, along the protoplasmic strands, entry being first made through a broken protoplast. To test this hypothesis, a series of experiments was set up to determine the rate of movement of the agent both upwards and downwards in the same plant. The rate of movement has been found already for the agents of some tomato virus diseases by Boning and this work has been noted in the earlier paper of this series. On the other hand, some workers (see Holmes (4, 5)) have found that, in other instances, the upper portion of the plant becomes diseased much earlier than the lower, the interval being, in the case of tobacco, one of some weeks. It is rather important in the light of this difference of opinion to establish whether or not movement is approximately at the same rate upwards and downwards in the stem of tomato. If the main movement of the virus were always in one direction (either up or down), then, presumably, there would be a prima facie case for the contention that movement was taking place along one of the organised conducting channels. If it be shown that movement tends to be upwards and downwards at apparently the same rates in a single plant, then the presumption is that the movement of the agent is not confined to any of the conducting systems.

The fact that symptoms normally appear at the top of the plant first need not, obviously, be taken as evidence of a greater movement in that direction. The presence of meristematic tissue, it is now generally considered, is necessary for the active multiplication of the virus agent. To avoid the difficulty attendant on this consideration, a series of experiments was set up, in which the axillary buds all along the stem were made available as the centres of active virus multiplication. In a typical experiment of this kind a plant was inoculated on a leaf which was rather more than half-way up the stem. The inoculation was made on the pinnae of the fifth leaf. After 3 days the plant was carefully cut up into sections with due precautions against accidental infection. In each section there was an axillary bud. The pieces of the stem were planted as cuttings. There were six cuttings, each with an axillary bud, the top and the root portions of the stem, the latter having the axillary bud of the first leaf. The leaves were numbered from below upwards. Within 11 days, the leaves of all the cuttings except those with the first and second axillary buds had developed symptoms of the disease. The distance travelled by

the agent in 3 days was, therefore, three internodes upwards as against two downwards—probably a difference so slight as to be ignored, though there is usually slightly greater movement upward than downward. In other experiments it was found that the virus agent had travelled downwards in the tomato plant 40 cm., *i.e.* down 20 cm. of petiole and 20 cm. of stem, in the case of large plants, in less than 6 days, while it had travelled upwards a slightly greater distance in the same time. In the controls of all the experiments, the upper leaves were always the first to show the symptoms, the leaves of the axillary buds only becoming mottled at a much later stage. These results, which confirm the data of Boning, seem to indicate that there is no rapid movement of the virus agent in any one direction in the plant, but rather that the agent moves slowly up and down the stem from the point of insertion of the inoculated leaf. The slight tendency to quicker movement upward may be associated with the increased rate of multiplication of the agent, which is usually found in meristematic tissues.

THE DEVELOPMENT AND MOVEMENT OF THE VIRUS AGENT IN THE PLANT IN THE DARK.

The effect of environmental conditions on the appearance of symptoms and on the development of virus disease in plants is considerable. The main difficulty, which inevitably suggests itself in connection with the interpretation of the results of experiments conducted under different environmental conditions, is that attendant on the impossibility of deciding between the specific effect of the conditions on the agent itself and on the host plant. Clearly, if the metabolism of the host plant is materially altered as a result of the altered environment, the effect on the development of symptoms, etc., might be considerable despite the fact that little alteration of the agent had taken place. In the following series of experiments the effect of new environmental conditions appeared to be slight or completely absent. Eighteen plants about 6 inches high were put into a darkened chamber on August 5th. On the 6th, 24 hours afterwards, they were inoculated with aucuba mosaic, each on the pinnae of the third leaf from the base. Six of them (Series A) were put back into the glasshouse, while the other twelve were put back into the darkened chamber. The inoculated leaf of each of the six plants in the light and of each of six of the plants in the dark was removed on August 10th, 96 hours after inoculation. Thereafter the six darkened plants were placed in the light (Series B). Symptoms appeared on all the plants—both those which had been continuously in the light and those which

had been kept for 4 days following inoculation in the dark—on August 13th and 14th. There was apparently little or no difference between the reaction of the control plants and the experimental plants as regards their response to virus disease. The other six plants were taken out of the dark chamber on August 11th—5 days after treatment (Series C). The treated leaves were removed. These plants, 24 hours later, showed signs of a "permanent wilt," and the older leaves, especially, were practically all dead, despite the fact that there was no suggestion of water shortage at the roots as shown by the absence of subsequent recovery in the light and on watering. The general appearance of the leaves is illustrated in Plate XX, figs. 1 and 2. On first inspection the appearance suggested a severe necrosis—although thorough examination revealed the fact that the condition of the leaves was typical of desiccation rather than of necrosis. Further work on this "permanent wilt" indicates that it is associated with the carbohydrate content of the leaves, either directly or indirectly, and the data which have been obtained will be given in a later paper.

For convenience, the details of this experiment are set out in tabular form in Table III.

Table III.

Series A	In dark 24 hours	Inoculated	In light thereafter
Series B	,, ,,	,,	In dark 4 days—in light thereafter
Series C	,, ,,	,,	In dark 5 days—in light thereafter

All these plants developed symptoms of aucuba mosaic.

The results so far obtained have shown that there is no evidence that darkness delays the spread of the virus agent in the plant, and that, the virus having spread, symptoms which subsequently appear in the light after the same period of inoculation are of the same type as those which appear in the normal plants. Symptoms do not normally appear in the dark on young plants, nor is this a matter for surprise when it is remembered that tissues of a young normal tomato plant collapse in the dark after 7–10 days' treatment. It must further be remembered that the general yellowing of the tissues of plants kept in the dark would preclude the recognition of mosaic symptoms. Actually, even this collapse of the older leaves is not sufficient to prevent completely the formation of symptoms which do appear on the younger leaves which subsequently develop if the plants are not kept in the dark for too long a period of time. In older plants, the tissues of which are sufficiently supplied with reserve material, etiolation takes place to a lesser or greater extent and the wilting is delayed, but, in the discoloured tissues which appear as a

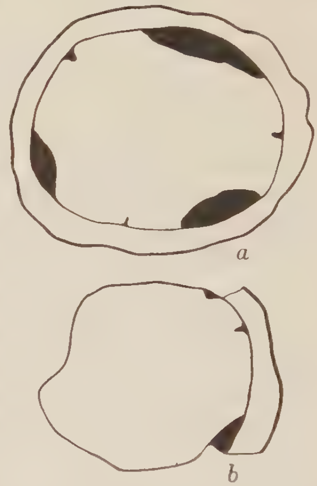
consequence of etiolation, the development of symptoms of aucuba mosaic is very difficult of assessment.

THE UPWARD MOVEMENT OF THE VIRUS AGENT IN STEMS AFTER THE REMOVAL OF PARTS OF THE VASCULAR TISSUE.

The evidence presented in this and in the previous paper appears to prove fairly conclusively that the xylem is not the normal path of movement of the agent of aucuba mosaic in tomato, and so far no evidence has been adduced to show that this agent differs radically from those of other virus diseases in this respect. The question of the actual path of movement, however, presents more difficulty. By analogy with the movement of metabolites, and from the experiments on the movement of the agent up and down the plant, it has been suggested that for this virus agent movement seems to be possible in any living cells. That is to say, the agent moves in the phloem rather because the tissue is composed of living cells than because it is transported passively, as are the carbohydrates for example. Some additional evidence which, it is thought, definitely supports this hypothesis, is here presented.

In the stem of the young tomato plant about a foot in height it is possible to see the position of the vascular strands in the internodes. When the plant is held in front of a bright light the strands appear as dark lines against the lighter ground tissue. The removal of portions of the vascular tissue in the internodes presents very little difficulty, therefore, and can be carried out without greatly disturbing the living cells of the cortex and medulla. In a series of plants of this kind portions of the bundles about half an inch long were removed all from one internode so that the continuity of the bundles in the plant was broken. As a consequence, the upper portion of the plants collapsed completely in a very short space of time, and the plants were not suitable for further experimentation. It was noticed, however, that there were in each internode three main bundles and three intermediate small bundles. When the three larger bundles were removed the three smaller appeared to allow of the passage of sufficient water to keep the upper leaves turgid. Similarly when all except one of the larger bundles were removed, that is, when the plant was ringed practically all the way round so as to expose the parenchyma of the pith, sufficient water passed up to keep the tissues above turgid. Various groups of plants were so treated and the damaged tissues heavily smeared with vaseline. The lower leaves were then rubbed with macerated virus material. After intervals of 3 or 4 days the upper portion of each of the plants was removed and planted as a

cutting. For convenience, before treatment the leaves of the middle portion were removed, as illustrated in Plate XVIII. Untreated plants, also with the leaves of the middle portion removed, were similarly inoculated and used as controls. It was found that symptoms appeared on the cuttings of the treated plants after the same interval of time—17–19 days—from the time of inoculation, as on those of the control plants. In another series the tops were not removed after inoculation, and the plants were set aside in the glasshouse to develop symptoms. Symptoms in this case developed on the control plants and on the treated plants after the same interval of time—10 days. In these experiments, again, aucuba mosaic in tomato was used throughout. Text-fig. 1 shows how much of the vascular tissue could readily be removed without substantially impeding the movement of the virus and it is suggested from a consideration of this data that there is strong presumptive evidence that the movement of the agent of aucuba disease of tomato can take place and does take place readily through any living tissue and that the phloem is, in this case, not the main channel of movement in the normal plant.



Text-fig. 1. Outline drawings (projection on same scale) of sections of tomato stems: (*a*) normal stem; (*b*) after removal of most of the vascular tissue (cf. Plate XVIII).

Summary.

In this paper the results of some experiments with aucuba mosaic in tomato are discussed. These results support the general thesis that the agent does not normally travel in the xylem stream. The movement of tobacco mosaic in tobacco and in tomato was found to be similar to that of aucuba mosaic. The majority of the experiments were carried out with aucuba mosaic in tomato.

It was found that filtered virus juice from virus-infected plants was readily absorbed at the cut end of a petiole and thence travelled into the xylem of the main stem. The removal of the treated petiole within 48 hours prevented infection taking place. On the other hand, when the petiole was left attached the experimental plant developed symptoms in the usual manner. This type of experiment was repeated with the

exception that, after the removal of the treated petiole, the leaves above were crushed. Infection of the plant followed this treatment. This experiment was combined with the earlier experiments in which the living tissue of an internode was killed by steam. The agent was found to be carried mechanically *in* the xylem across the dead tissue.

As a consequence of this observation the experiments with plants with "steamed" internodes were repeated. It was found that in no case did crushing of the leaves induce symptoms on the upper part of the plant when inoculation had been made on the lower side of the "steamed" internode.

It was concluded that the virus agent did not normally enter the water stream, and when it was introduced experimentally into it, though it was carried round, there was no mechanism by which it could leave the vessels.

The absence of the agent from the hydathode exude was demonstrated.

Apparently the agent cannot enter an unbroken cell, nor can it move through or out of dead cells. It has been found that great care must be taken to ensure the absence of traces of toxic substances from inocula to be tested, otherwise infection may not take place even in the presence of the agent itself.

The rates of movement of the virus agent in the tomato are practically the same upward or downward. The slightly greater rate of upward movement appears to be associated with the greater metabolic activity which occurs in the upper portion of the plant. The movement of the virus agent along the protoplasmic strands has been examined by inoculating plants with infective juice after the removal of large portions of the vascular tissue. This treatment does not appear to delay the movement of the agent up the stem.

In a final group of experiments, darkness did not appear to have any effect on the multiplication of the virus in the tissues. Too prolonged periods in the dark, however, caused the permanent wilting of both diseased and healthy plants. This "wilting" is considered as being due to the respiration of carbohydrates, etc., and the earlier collapse of the diseased plants as being due to a smaller carbohydrate supply in them.

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EXPLANATION OF PLATES XVII–XX.

PLATE XVII.

Tomato plant with "steamed" internode, after the absorption of eosin by a petiole on the lower part of the plant.

PLATE XVIII.

(*a*) A control plant, with a few leaves removed and inoculated with aucuba mosaic below; (*b*) plant with vascular tissue of one internode removed (at *x*). Both plants developed symptoms of aucuba mosaic in the upper parts after the same period. In (*b*) note the development of the axillary shoots in the lower part of the plant.

PLATE XIX.

Fig. 1. The leaves of a young tomato plant with drops of water exuded from the hydathodes.

Fig. 2. Photomicrograph of the tip of a young tomato leaf showing bundle end and simple type of water stoma.

Fig. 3. Mass of adventitious roots developed above the "steamed" portion of a tomato stem.

PLATE XX.

Fig. 1. (*a*) Diseased plants (aucuba mosaic); (*b*) healthy plants. Both sets photographed after 5 days in dark chamber.

Fig. 2. "Healthy" plants: (*a*) after fortnight in dark, with well-watered soil; (*b*) control, kept in light, with dry soil.

(*Received January* 10*th*, 1931.)

CALDWELL.—THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS (pp. 279–298).

CALDWELL.—The Physiology of Virus Diseases in Plants (pp. 279–298).

Fig. 1.

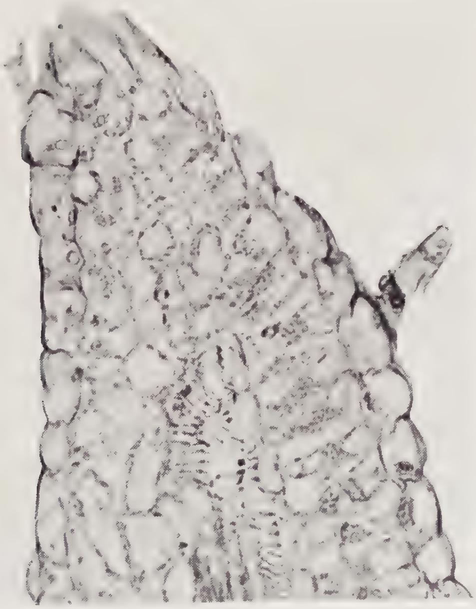

Fig. 2.

Fig. 3.

CALDWELL.—The Physiology of Virus Diseases in Plants (pp. 279–298).

Fig. 1.

Fig. 2.

CALDWELL.—The Physiology of Virus Diseases in Plants (pp. 279–298).

ON THE TRANSMISSION OF POTATO LEAF-ROLL BY APHIDES

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(With Plate XXI.)

Introduction.

In recent years an increasing amount of interest has been attached to the precise rôle played by insect vectors of potato leaf-roll. This has been stimulated by the close relation shown by Kenneth Smith (1) to exist between one species of aphis (*Myzus persicae* (Sulz)) and the disease. This worker has obtained a high percentage of successful transmission with this species, but only negative results with other aphides including *Macrosiphum gei* (1).

During the winter of 1928–29, however, and before the publication of Smith's paper, some results obtained in this College (2) had suggested that *Myzus circumflexus* (Buckton) and/or *Macrosiphum gei* (Koch) could, in fact, act as very efficient vectors. In this experiment two varieties of potato (Arran Comrade and Kerr's Pink) were infested with a mixed lot of aphides, consisting so far as could be ascertained solely of these two species of aphis[1], with the result that abundant evidence of transmission was secured. When the plants were first infested the bulk of the aphides consisted of *Macrosiphum gei*, but *Myzus circumflexus* proved to be much the more prolific and the relative numbers were reversed on the completion of the work. Although no specimens of *Myzus persicae* were found in the samples examined, the possibility of their presence in very small numbers could not be ignored, so that critical work was immediately begun with all three species.

Experiments in 1929.

Six individuals of each of the three species *Myzus persicae*, *Myzus circumflexus* and *Macrosiphum gei* were identified by Dr Davies, and were multiplied under strictly isolated conditions, since it was recognised that the validity of the results depended on the certainty that no ad-

[1] For the identification of aphides in this and subsequent experiments the writer is indebted to his colleague, Dr W. M. Davies.

mixture of aphides was possible. Twelve plants each of the varieties Arran Comrade and Kerr's Pink were used for each species of aphis. The tubers were first thoroughly scrubbed and then dipped in nicotine sulphate to ensure that no aphides were carried over in this way. The experimental plants were grown from half-tubers planted in separate insect-proof glasshouses, and each plant was infested by four apterous females of one or other species. The corresponding half-tubers were planted in another glasshouse as controls.

Of the 24 plants infested with *Myzus persicae*, 4 Kerr's Pink and 5 Arran Comrade plants showed symptoms of leaf-roll; the respective numbers for the plants infested with *Myzus circumflexus* were 7 and 5, whilst there was one case of transmission by *Macrosiphum gei*. On planting the progeny of the "persicae" group, 12 out of 38 developed leaf-roll; 15 out of 40 tubers in the "circumflexus" group, and the two tubers from the infested "gei" plant also showed definite leaf-roll symptoms.

The control half-tubers, however, fared badly in a glasshouse in which the heating became inefficient and plant growth was distinctly abnormal. No leaf-roll was seen amongst these controls, but under the circumstances a repetition was advisable under more favourable conditions for growth.

Experiments in 1930.

The same three species of aphis were used to infest, in this case, the variety President. Every precaution was taken to ensure that aphides were not carried by the tubers and that none but the experimental aphides, raised under strictly controlled conditions, could obtain access to the plants. The possibility that a virus carried without symptoms might affect the development of leaf-roll was taken into account by the use of duplicate plants in each case, one of which was from a proved virus-free stock and the other from a specially selected symptomless stock which, however, had not been tested for the presence of a hidden virus. It may be said at once that both stocks reacted alike throughout the experiment. The half-tuber controls showed normal growth and remained perfectly healthy. Six plants were used for each species of aphis, ten apterous females being placed on each plant 5 weeks after planting to ensure the full development of symptoms in the current year. On the completion of the period of infestation the plants were fumigated, kept under observation for a day or two and then removed to the same house to mature.

The first signs of rolling occurred in the "persicae" group 24 days after infestation. A further 16 days, or 40 in all, elapsed before rolling

was seen in the "circumflexus" group, whilst of the plants infested with *Macrosiphum gei* only one had suspiciously inrolled leaf margins after 56 days. Plate XXI, figs. 1 and 2, illustrate this curious lag in the development of symptoms in the "circumflexus" group, one being photographed when rolling had only just begun in the youngest leaves although it was well marked in the "persicae" plants, and the other when the full symptoms had developed 1 month later. All the plants of these two groups developed leaf-roll and there was little or no difference in the severity of the symptoms.

A red colour developed between the veins on the underside of the leaves concurrently with the rolling, and was of equal intensity in the first two groups, but with *Macrosiphum gei* only two plants showed the faintest trace of colour in an unfolded bud. Similarly, there was a stimulation of growth in the usually dormant axillary buds, which in the plants infested with *Myzus persicae* or *Myzus circumflexus* grew to sturdy laterals equalling the main shoot in length in some cases. The plants infested with *Macrosiphum gei*, on the other hand, showed some growth of laterals but not nearly so much as in the other two groups.

On planting the progeny under aphis-proof conditions, the tubers from the suspected "gei" plants developed normally, so that one could say with confidence that no transmission had occurred with *Macrosiphum gei*. With *Myzus persicae*, however, 53 per cent. of the progeny which grew produced leaf-roll plants, as did also 45 per cent. of the viable progeny of the *Myzus circumflexus* plants. There was therefore ample confirmation of the power of these two species to transmit leaf-roll[1]. A considerable number of tubers in all three groups failed to grow, and at first this was suspected to be due to abnormally heavy infection. Since, however, the number of failures was almost as large in the "gei" group (9 out of 39) as in the "persicae" group (11 out of 30), whilst only 6 tubers in the "circumflexus" group failed out of 34, this view was abandoned, for it is difficult to believe that all infected tubers in the "gei" group failed to grow.

MYZUS CIRCUMFLEXUS AS A VECTOR OF LEAF-ROLL.

In letters to *Nature* (3, 5) announcing the discovery of this new vector[2], the writer pointed out that since it has not been recorded on potatoes in the field it is unlikely to be of importance in spreading leaf-roll under these conditions. Yet it must not be ignored, especially by growers

[1] The presence or absence of leaf-roll was in all cases confirmed by Sach's iodine test.

[2] This has since been confirmed by Dr K. M. Smith. (*This Journal*, XVIII, No. 2, 1931.)

attempting to raise healthy stocks out-of-doors, since it has been recorded in the field on oats and certain clovers. Academically it is of interest in that the ease with which the apterous female can be identified should make it a valuable species to use in critical transmission work. The opinion was also expressed that there is a growing tendency to assume a specific and unique relationship between *Myzus persicae* and leaf-roll—a tendency which requires correction since the relationship, whatever it may be, is shared to some extent by another species. The point is not a trivial one, particularly in view of the fact that other species have been found occasionally to transmit leaf-roll, *e.g. Macrosiphum gei* (this paper, and Murphy and M'Kay(6)) and *Myzus pseudosolani* (Murphy and M'Kay(6) and Elze(7)). Indeed both these workers report that insects quite distinct from aphides act, at least occasionally, as leaf-roll vectors. If such an unique relation between the disease and one species of insect did exist it is easy to imagine the superstructure which might be built upon a supposed analogy with certain other virus diseases.

As regards reliability in transmitting leaf-roll and the intensity of the symptoms displayed, there is little doubt that *Myzus circumflexus* is as efficient as *Myzus persicae*. The lag in the development of symptoms when the former species was used, however, is difficult to interpret. It was not observed in the 1929 experiments, but this in no way casts doubt on the reality of the observation in 1930, for the cause—whatever it may be—is not necessarily present in all specimens or in the same individual in all seasons of the year. It suggests a temporary retardation in the activity of the virus, for which a possible explanation may be found in K. M. Smith's statement that *Myzus circumflexus* possesses a toxin in its saliva capable of producing pseudomosaic symptoms in Solanaceous plants including the potato(4). The writer has not observed this false mosaic, but this is possibly because the species has only been used when already carrying the leaf-roll virus, and its effect when non-viruliferous may be quite different.

The necessity for some development of the virus in the body of the aphis, the facilities for which might differ in various species, seems to be excluded by the fact that leaf-roll is transmissible by grafting without the agency of aphides at all. On the other hand there would appear to be an intimate relation between active metabolic processes and the leaf-roll virus, not fully shared by other viruses, since it has not been transmitted by needle inoculation, and only with considerable difficulty by grafting into semi-dormant tissues such as a tuber, whereas viruses of the mosaic group are usually transmitted readily by both methods.

Yet another possible explanation of the lag in symptom development is that less inoculum is introduced by *Myzus circumflexus* than by *Myzus persicae*. Some such effect of dosage is certainly suggested by the grades of rolling and stunting in infected plants of the same variety under field conditions. In the writer's experiments both in 1929 and in 1930, which are described above, an effort was made to ascertain whether such dosage effects occur. The plants in 1929 were divided into three groups which differed only in the length of the infestation period, viz. 1, 3 and 5 weeks. There was a small but definite increase in the severity of symptoms shown in the plants infested for the longer periods, both with *Myzus persicae* and *Myzus circumflexus*. No such tendency was noted in 1930, when the infestation periods for the three groups of plants were 2, 4 and 6 weeks respectively. Much further work is desirable, but it is at least evident that ten individuals, as used in 1930, give a sufficient dose to produce severe symptoms, and an increase in number of vectors beyond this does not intensify those symptoms. In 1929, however, only four aphides were used per plant, and it may well be that a range of symptoms might be found by still further varying the period of infestation with a smaller number of vectors.

Summary.

1. Evidence is submitted to show that *Myzus circumflexus* (Buckton) is as efficient a vector of potato leaf-roll as is *Myzus persicae* (Sulz). The implications, both practical and academic, of this fact are discussed, particularly in view of the retardation in the development of symptoms shown by plants infested with *Myzus circumflexus* carrying the leaf-roll virus.

2. *Macrosiphum gei* (Koch) only transmitted the disease once and its importance as a field vector is still an open question.

The writer is glad to acknowledge the help given by the laboratory assistant, Mr G. L. Turner, in the above work.

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EXPLANATION OF PLATE XXI.

Figs. 1 and 2 show the retardation of symptom development in plants infested with *Myzus circumflexus*. Fig. 1 photographed when secondary symptoms are obvious in plants infested with *Myzus persicae* and primary symptoms just beginning in the "circumflexus" plants. Fig. 2 shows well-developed secondary symptoms with both species. Infestation period = 4 weeks.

Figs. 3, 4 and 5 show typical symptoms with *Myzus persicae* (Fig. 3), and with *Myzus circumflexus* (Fig. 4); the absence of transmission with *Macrosiphum gei* (Fig. 5). Infestation period = 2 weeks.

(*Received February* 12*th*, 1931.)

Fig. 2

Fig. 5.

Fig. 4.

Fig. 1.

Fig. 3.

WHITEHEAD.—ON THE TRANSMISSION OF POTATO LEAF-ROLL BY APHIDES (pp. 299–304).

EXPERIMENTS ON THE CONTROL OF TOMATO LEAF MOULD (*CLADOSPORIUM FULVUM*) BY FUNGICIDES AND FUMIGANTS

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These experiments were begun because of repeated enquiries by growers concerning the control of tomato leaf mould by fungicides and fumigants. Their aim was to discover those substances which would control the disease on pot plants so that these might be recommended to growers for trial on commercial nurseries. Previous investigations on the control of this disease have been reviewed by Guba (1) in a recent paper and will not be discussed here.

Fungicides.

Protective action of fungicides.

To determine the protective action of fungicides, plants, previously sprayed with the fungicide and allowed to dry, were gently inoculated with a heavy suspension of spores in water, placed in a moist chamber for three days, and subsequently grown in a glasshouse. Controls received similar treatment except that they were left unsprayed or were sprayed with a spreader only. Five plants, each about 10 inches high, were used in each trial, and the spores were taken from fresh diseased leaves.

Spreaders. In early experiments saponin and soft soap were used as spreaders. Most of the fungicides to which saponin was added gave but partial control of the disease (see Table II). Soft soap was considered unsuitable for use on tomatoes because it hardened the foliage.

In later experiments Agral I was used as a spreader. The results of one preliminary trial are given in Table I and show that colloidal sulphur A controlled leaf mould more effectively when Agral I was added instead of saponin or soft soap, and that Agral I (0·25 per cent.) partly checked the disease on control plants.

In view of these results Agral I was used as a spreader in all subsequent work. The concentration employed was 0·15 per cent. because higher concentrations caused curling, and sometimes scorching, of the foliage.

Table I.

Effect of spreader on the control of leaf mould by colloidal sulphur A.

Conc. of fungicide and spreader (%)		Leaf mould attack
0·5 colloidal sulphur A	0·25 Agral I	Negligible
,, ,,	0·02 saponin	Fair
,, ,,	0·50 soft soap	,,
Control	0·25 Agral I	,,
,,	0·02 saponin	Severe
,,	0·50 soft soap	,,
,,	Unsprayed	,,

Results.

The results obtained are summarised in Table II. Each experiment was repeated twice at least, and in all experiments the controls developed leaf mould severely except those sprayed with Agral I.

Table II.

Control of leaf mould by fungicides.

Fungicide	Conc. (%)	Control: With Agral I	Control: With saponin
Ammonium copper carbonate	0·50	+ + + +	+ + +
,, polysulphide	0·40	+ + +	.
,, ,,	0·50	.	+ +
Calcium bisulphite	1·00	+ + +	+ +
Colloidal Bordeaux mixture	1·00	.	+ +
,, sulphur A	0·40	+ + + +	.
,, ,,	0·50	.	+ + +
,, sulphur B	0·40	+ + +	.
,, ,,	0·50	.	+ +
Lime sulphur	0·40	+ + +	.
,,	0·50*	.	+ +
Liver of sulphur	0·25*	+ + +	+ +
Salicylanilide	0·25*	+ + + +	+ + +
Sodium polysulphide	0·50	.	+
Solbar	1·00, 2·00*	.	+

* Slight scorch. No asterisk denotes absence of scorch.
+ = poor; + + = fair; + + + = fairly good; + + + + = good.

The results show that most fungicides gave better control of leaf mould when Agral I was added as a spreader instead of saponin. Effective control was obtained by spraying with ammonium copper carbonate, colloidal sulphur A and salicylanilide[1], while fairly effective control resulted on plants sprayed with ammonium polysulphide, calcium bisulphite, lime sulphur, liver of sulphur and colloidal sulphur B. Sodium polysulphide and Solbar gave poor control in early experiments and were not retried with Agral I as a spreader.

Preliminary trials have been made at commercial nurseries with some

[1] See Fargher, R. G., Galloway, L. D. and Probert, M. E. (1930). The inhibitory action of certain substances on the growth of mould fungi. *Shirley Inst. Mem., Didsbury*, IX, 37–52.

of the promising fungicides. Occasionally the plants were slightly scorched by liver of sulphur, salicylanilide, lime sulphur and ammonium polysulphide, but ammonium copper carbonate and colloidal sulphur A were non-injurious and were considered by growers to be suitable for use on tomato crops. These trials will be continued in 1931.

Observations at nurseries from 1927 to 1930 show that the disease appears towards the end of April and often becomes severe in May and June. During this early part of the season the favourable environmental conditions for leaf mould which obtain in the glasshouses can scarcely be avoided because the closely planted, free-growing crop must be watered frequently and because only a limited amount of ventilation can be given owing to unfavourable climatic conditions. It is during this period that spraying will probably prove most useful to the grower. From July to September ample ventilation is usually given and this is often sufficient to prevent serious attacks of leaf mould provided that the disease has not become severe during May and June.

The interval between successive sprayings must be determined by trials and will depend upon whether conditions are favourable or unfavourable for the disease. At present an interval of two weeks is suggested during May and June after which less frequent treatment will probably suffice to control the disease. Particular attention should be paid to those well-known areas of the nursery where leaf mould first appears and from which it spreads to the rest of the crop.

Curative action of fungicides.

Following upon the results obtained in the foregoing experiments, the curative action of the fungicides given in Table II was tested, using the same concentrations of fungicide and Agral I as those noted in the table. The standard procedure was to clamp approximately equal-sized areas of diseased leaf tissue in a vertical position and to spray each of them for ten seconds with 25 c.c. of the fungicide. When the tissue was quite dry some of the spores remaining on it were transferred to a weak glucose solution and tested for germination. Spores from the controls, which were sprayed with spreader only, germinated excellently.

The results in repeated trials were not always consistent, but from the evidence obtained it was concluded that ammonium copper carbonate and ammonium polysulphide were the most toxic since they usually killed about 95 per cent. of the spores.

These two fungicides have been used to spray diseased crops at large nurseries, but most growers preferred ammonium copper carbonate to

ammonium polysulphide because the latter left a little deposit on the fruit and discoloured the paintwork of the houses.

In one careful test ammonium copper carbonate was used in a glasshouse containing one thousand severely diseased plants, five hundred of which were sprayed three times at fortnightly intervals with 0·50 per cent. ammonium copper carbonate in 0·15 per cent. Agral I. The treatment checked the disease considerably. It is believed that the success of this experiment was due to the very thorough application of the fungicide. It is worth noting that a fresh growth of the fungus appeared around the old diseased spots seven days after each application.

Fumigants.

In these experiments the plants were first inoculated with a spore suspension and allowed to dry. They were then exposed to the vapour of the fumigant for two hours in a dry, glass chamber (capacity 2·7 cubic feet and temperature 17° C. to 20° C.) in the laboratory, placed in a moist chamber for three days and subsequently grown in a glasshouse. Five plants, each about ten inches high, were used in each trial, and the fumigants were vaporised by heating with a small flame. Controls were inoculated but were not exposed to the vapour, and they invariably developed leaf mould. During the trials, diseased leaves and glass slides dusted with spores were also placed in the fumigating chamber, and spores from these were tested for germination the day after fumigation.

Table III includes those fumigants which failed both to control leaf mould and to kill the spores on diseased leaves and glass slides. When used in amounts greater than those given in the table, the fumigants injured the plants yet frequently failed to kill the spores. Sometimes the spores were killed on the glass slides but not on the diseased leaves.

Table III.

Fumigants which failed to control leaf mould.

Fumigant	Amount (oz. per 1000 cu. ft.)	Fumigant	Amount (oz. per 1000 cu. ft.)
Acetophenone	1·30	Iodobenzene	1·00
Amyl alcohol	2·60	Methyl salicylate	1·30
Anthraquinone	2·60	Nitrobenzene	1·30
Bromobenzene	0·26	Nitronaphthalene	0·20
Chloranil	2·00	*o*-nitrotoluene	0·65
o-cresyl-methyl ether	1·30	*p*-nitrotoluene	1·30
p-dichlorbenzene	2·00	Phenyl salicylate	0·20
Dimethyl-aniline	1·30	Pyrocatechol	0·19
Epichlorhydrin	0·32	Quinoline	1·30
Ethyl benzoate	0·65	Resorcinol	1·30
Ethyl formate	3·91	Terpinol	1·30
Hydroquinone	2·60	Tetrachlorethane	1·30

The fumigants shown in Table IV prevented leaf mould from developing on inoculated plants and also killed the spores on both diseased leaves and glass slides.

Table IV.

Fumigants which controlled leaf mould.

Fumigant	Amount (oz. per 1000 cu. ft.)	Leaf mould attack	Plant injury
Ethylene dibromide	2·60	Negligible	Stunting and hardening effect sometimes
Quinone	0·26	,,	Nil
Thymol	0·45	,,	,,

In later experiments quinone and thymol were tested under various environmental conditions in a glasshouse of 635 cubic feet capacity, and it was found that both compounds scorched the plants when used in amounts which caused no injury in laboratory experiments. Smaller amounts were then tried, but with thymol scorching occurred at concentrations which failed to control the disease. On the other hand, quinone at a concentration of 0·14 oz. per 1000 cubic feet prevented leaf mould from developing on inoculated plants yet caused little or no scorching. In addition it killed the spores on glass slides but not those on diseased leaves. At a concentration of 0·08 oz. per 1000 cubic feet quinone failed to control leaf mould. On account of the high price of quinone experiments have been made with a cheaper product, and the results indicate that this product may be as effective as pure quinone.

The above experiments show that quinone vapour is very toxic to the spores of *C. fulvum*. Further work is necessary to ascertain if quinone fumigation is practicable for the control of tomato leaf mould in large glasshouses.

Sulphur vapour. In these experiments the plants were inoculated with a spore suspension only on the lower side of the leaves, and when dry they were placed in a glasshouse of 635 cubic feet capacity in which 2 oz. of flowers of sulphur were vaporised by Campbell's apparatus. Fumigations were carried out late in the afternoon, and next morning the plants were removed from the glasshouse, placed in a moist chamber for three days and subsequently grown under conditions favourable for leaf mould. Control plants were inoculated but were not exposed to the vapour, and they always developed the disease. Many trials were made, but in none of them did ignition of the sulphur occur.

Experiments were made in which the temperature at the time of fumigation was 19° C., while in others it was 27° C. In some trials the

humidity was high, in others it was low. Almost identical results were obtained under these very different sets of atmospheric conditions.

The results showed that plants in inverted positions during fumigation remained almost healthy, while plants in upright positions during fumigation developed a severe attack of leaf mould. Similar results were obtained in later trials in which the plants were inoculated after, instead of before, fumigation. The results are explicable in that the inoculated (lower) side of the leaves of inverted plants was covered with sulphur, while that of upright plants received little, if any, sulphur.

From these and other results it was clear that plants in upright positions during fumigation were protected from infection only on the upper side of the leaves. Attempts were made, therefore, to determine on which leaf surface infection occurs under natural conditions. In discussing the results of certain inoculation experiments Guba (1) states that "leaf infection is almost altogether hypophyllous." An experiment was made by the writer in which sixty small drops of a spore suspension were placed on the upper and lower sides of leaves, and the results showed that the number of infections was fifty-eight and fifty-nine respectively. It was also interesting to find that all the leaves inoculated on the upper side developed leaf mould only on the lower side. Thus the fact that the disease almost always occurs on the under side of leaves does not necessarily mean that infection takes place there. Efforts to ascertain on which leaf surface infection occurs under natural conditions were not successful and they are being continued.

It is worth noting that in the above experiments with sulphur vapour the spore pustules on the under side of the leaves of diseased plants in upright positions were not killed by the vapour.

Fumigation of empty glasshouses.

It has been shown elsewhere (2) that the spores of *C. fulvum* may overwinter on both glasshouse structures and diseased leaves, and that such spores may cause infection in the spring. Experiments were made, therefore, to ascertain if houses may be freed from infection by fumigation during the winter. The trials were made in a glasshouse of 25,000 cubic feet capacity, and the house was closed for about twenty hours from the commencement of fumigation. Seventy diseased leaves and thirty glass slides dusted with spores were placed in positions on the floor, roof and sides of the house, and spores from these were tested for germination the day after fumigation. Control spores, kept in a neighbouring glasshouse, germinated excellently. As a result of preliminary trials in a small glass-

house formaldehyde, cresylic acid and sulphur dioxide were selected for the experiments.

Formaldehyde. The gas was generated by pouring a 40 per cent. aqueous solution of formaldehyde upon crystals of potassium permanganate. Each of four deep receptacles was charged with 1·25 pints of formalin and 0·8 lb. of potassium permanganate. The weather was fine but dull, and at the time of fumigation the temperature was 24° C. and the humidity 85 per cent. Germination trials showed that practically every spore was killed by the treatment, which cost about six shillings. When half the above quantities were used spores in exposed places were killed but not those in crevices and in pipe hooks.

Cresylic acid. One pint of pale straw cresylic acid was vaporised by each of two naphthalene lamps (Monro's). The results showed that the spores were killed in some parts of the house but not in others, and suggested that the vapour was irregularly distributed.

Sulphur dioxide. This gas was generated by burning flowers of sulphur. Preliminary trials in a small glasshouse of 635 cubic feet capacity showed that quick generation of the gas was essential. For example, 1 lb. of sulphur in one heap failed to kill the spores, whereas four heaps, each of 0·25 lb., burned simultaneously, killed almost every spore. Various experiments were made under very different environmental conditions and almost identical results were obtained.

In an experiment made in the large house of 25,000 cubic feet capacity five heaps of sulphur each of 5 lb. were burned simultaneously, and the result showed that almost every spore was killed. The fumigation, which cost about three shillings, was carried out on a rainy day and the temperature at noon was 15° C. When half the above quantity was used the spores were killed except on two leaves near the apex of the roof.

The foregoing experiments indicate that large empty glasshouses may be freed from infection by fumigation with formaldehyde or sulphur dioxide. It is, perhaps, impracticable to fumigate large, unpartitioned blocks of houses, but in propagating houses or in houses where it is desired to replant immediately an area carrying a badly diseased crop, fumigation should be carried out in order to lessen the risk of infection.

Summary.

1. Experiments have been made on the control of tomato leaf mould (*Cladosporium fulvum*) by fungicides and fumigants. The results showed that most fungicides gave better control of the disease when Agral I was added as a spreader instead of saponin. Effective control was obtained

on pot plants sprayed with ammonium copper carbonate, colloidal sulphur A, and salicylanilide. Preliminary trials on commercial nurseries showed that ammonium copper carbonate and colloidal sulphur A were suitable fungicides for use on glasshouse tomato crops. Further work showed that ammonium copper carbonate was useful for spraying diseased tomato crops.

2. The disease was controlled by the fumigants ethylene dibromide, quinone, and thymol, in laboratory experiments, and by quinone in trials made in a small glasshouse. Further work is necessary to ascertain if quinone fumigation is practicable for the control of tomato leaf mould in large glasshouses.

3. Fumigation with Campbell's sulphur vaporiser protected only the upper side of the leaves from the disease and also failed to kill the spores on the under side of diseased leaves. Experiments made to determine on which leaf surface infection occurs under natural conditions were not successful, but inoculation tests showed that infection may occur on either side of the leaf.

4. It has been shown that large empty glasshouses may be practically freed from infection by fumigation with formaldehyde or sulphur dioxide.

The writer wishes to express his thanks to Dr W. F. Bewley for suggestions and advice during the course of the investigation.

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THE HISTOGENY OF POTATO SCAB

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(With Plates XXII and XXIII and 3 Text-figures.)

The subject of common scab of potatoes is one which has engaged the serious attention of mycologists for upwards of a century, but so far the causative organisms of the disease have not been satisfactorily demonstrated in the *living* tissue of the host plant, and consequently their mode of entry into potato tubers has remained an assumption rather than an established fact. It is not surprising, therefore, that little is known of the origin and growth of the scabs—a point which is of peculiar interest in view of the resistance that certain potato varieties are stated (14) to offer to the disease and in view of the marked differences in external appearance of the several types of scab described by Millard and Burr (6) and by Wollenweber (13) and stated by them to be caused by distinct *Actinomyces* species. The aim of the present work is to trace the origin and development of a "pitted" type of scab which results from the activities of *Actinomyces scabies* (Thaxter) Gussow, emend. Millard and Burr, and to compare its structure with that of a raised type of scab, the "tumulus" type caused by *Actinomyces flavus* (Millard and Burr), in order to ascertain whether the differences in appearance of these scabs are due to marked differences in internal structure. It was also hoped that it would be possible to demonstrate the presence of the causative organisms within infected tuber tissues and to indicate means whereby the pathogenicity of *Actinomyces* species to plant tissues may be demonstrated microscopically. For these purposes as many stages of the two types of scab as it was possible to obtain were microtomed and examined after staining with Sudan III to demonstrate the presence of suberised tissue, and with Delafield's haematoxylin to make evident the organisms within the tissues.

The pitted type of scab.

When the exteriors of lightly or moderately scabbed tubers are scrutinised carefully, small brown-tinted spots can be found in the apparently unscabbed areas. These spots, some of which are approximately circular in outline and no more than 1 mm. in diameter, are the

earliest visible stages of scab and are very similar in appearance to lenticels. More mature scabs differ from them in shape and in size and are of two kinds; those that are isolated and may be called simple, and those that are joined making a compound scab. The isolated scabs are more or less circular in outline, having a sunken centre which is sometimes distinctly, and at other times scarcely, visible, whilst the compound scabs may retain separate the sunken centres of their component parts, or these centres may link up to give a simple or branched furrow. The isolated scabs vary from 2 to 8 mm. in diameter when the whole of the browned area of the scab is included, and the depressions in the centres of these areas rarely exceed 5 mm. in diameter. On the other hand, with compound scabs, the whole of the tuber surface may be included in the browned area or only a portion of its surface may be so covered. The furrow of this kind of scab is usually from 1 to 5 mm. in diameter.

Microtome sections were made of all stages of the two types of scab just mentioned, and their development was traced from the earliest stages to that in which growth was arrested by the lifting and maturing of the tubers.

The earliest stages of "pitted" scab.

As previously mentioned, a scab at its inception appears to be a lenticel when viewed from the exterior, and when cross-sectioned is usually of the kind shown in Plate XXII, fig. 1, where a young infected lenticel of recent formation is depicted. Most of the cells in its upper half are elongated radially and are arranged in rows which originate in the curved meristematic area at *AA*. The elongated cells are closely packed without air spaces, such as are found between the loose-fitting rounded cells of wider and more mature lenticels, and are unsuberised. All the cells external to the dotted line contain branching fungal hyphae which unfortunately could not be brought out distinctly in the photograph. No signs of any suberised protective barrier could be found beneath this infected area.

At the exterior, where the elongated cells have ruptured the periderm, there is a cap of dark staining material, made up of collapsed cells and their contents, amongst which mycelial threads of the fungus ramify. It is these cells that colour the young scabs brown, for when untreated with a stain they are found to have brown tinted walls. Starch grains are missing from the five or six layers of cells immediately beneath the meristem of the scab, and, for some distance around it, very little starch can be found in the cells abutting on the periderm.

A later stage of scab is shown in Plate XXII, fig. 2, which is a cross-section of a larger and flatter lenticel-like structure than that of Plate XXII, fig. 1, but is made up, similarly, of a mass of elongated cells which have arisen from a meristem below. The lower half of the band of elongated cells extending beneath the gap in the periderm, and joining with it in the region of its exposed edges, show darker walls in the photograph than the other tissues because they alone retained safranin after contrast staining with safranin and light green. In unstained sections these cells have brown-tinted walls. When treated with Sudan III they do not stain red, and hence, in spite of retaining safranin, they cannot be regarded as properly suberised. The periderm on the other hand showed strong suberisation. At the exterior, and capping the elongated cells, is a mass of collapsed tissue, where *Actinomyces* threads may be found, and which at A appears to be continuous with, and a part of, the periderm, but since none of these collapsed cells gave a suberin reaction they cannot belong to the periderm. There is thus in this scab as yet no suberised barrier beneath the infected area. At B some of the elongated cells also have collapsed, contributing thereby to the size of the cap, and have led to the formation of a slight depression, the forerunner of the distinct furrow or hollow that is found in mature scabs of this type.

From the foregoing examination of the first two of the young scabs described, it is evident that the fungus can infect young lenticels and bring about browning and collapse of some of their cells, but no protective suberised tissues are erected at this stage, by the host, beneath the invaded area.

Mature scabs.

More mature scabs differ from those already described in that they have destroyed more of the hosts' tissues and in so doing have obliterated all trace of the route by which they entered the tuber. They also differ in that the infected portion is isolated from the healthy tissue beneath by means of a cork barrier.

Plate XXII, fig. 3, is a cross-section of a mature isolated scab, in the centre of which there are a number of cells, some having dark-tinted walls only and others having dark-tinted contents in addition. In all of these cells the tinting is due to the brown stain that is found in some cells of all stages of scab. Within the dark-stained tissue of this particular scab are compact masses of branching mycelia of the fungus-bearing spirally coiled sporophores which, because the section was unstained, are not

visible in the photograph. Beneath the infected portion of the scab and closely following the inner contours of this diseased tissue, is a cork barrier *AA* which has arisen from a meristem that lies upon its inner surface. Some six layers of well-suberised, regular-shaped cells arranged in rows have been cut off from this meristem, and in addition, as at *B*, elongated cells frequently terminate the rows. Usually the elongated cells have thickened walls, excepting the inner tangential walls, which are brown tinted owing to a deposit of a brown protein-like substance occurring upon them. Sometimes a thin deposit of suberin is present on their inner surfaces, but the deposit is not found to be heavy or to be uniformly distributed over these surfaces. That each elongated cell terminates a row of wound cork cells suggests it is either the external portion of a cortical cell of which the inner portion has given rise to the remainder of the row, or, more probably, it is the first cell of a row of cells cut off from the enlarged lenticel meristem. In either case it is evident that suberisation of the elongated cell cannot precede cell division in the row of cells of which it is a member.

Since in this scab there are no signs of a suberin block situated external to the wound cork, and of the type described by Priestley and Woffenden (10) and stated by them to be essential to the formation of wound cork, it is evident that the wound cork of this scab is not due to the same sequence of events that they describe in ordinary wound healing in the potato tuber. This fact is rather surprising, but, as will be pointed out later, it is substantiated not only by an examination of all stages of scab in which wound cork is present, but also by examination of a potato sprout infected by the fungus. In the youngest scabs also it has already been shown that no suberin block precedes cell division in their tissues.

The manner in which a scab increases in size after it has reached the stage of Plate XXII, fig. 3, is shown in Plate XXII, fig. 4, where there is depicted a compound furrowed scab in cross-section. The furrow is more marked than that of the previous scab, and is already extending beneath the first protective cork barrier, erected at *A*, by three lines of invasion at *B* and *C* and *D*. In each of these areas is a central mass of large cells, evidently cortical cells from their shape and the starch grains they contain. Partly surrounding each mass is a curved barrier of cork cells, some three or more layers deep, forming a more or less continuous band beneath the infected areas and linking up, more or less completely, at its extremities with the tuber periderm. Each of these barriers has arisen not from a regular meristem but from meristematic divisions of the

cortical cells as at *E*. One important result of the erection of these barriers is that fresh areas of the tuber are cut off from internal food and water supplies and consequently disintegrate, thus adding to the size of the scab furrow. If the cells of the cork barriers are examined carefully it is found that they are similar in appearance and arrangement to those of the barrier beneath the scab of Plate XXII, fig. 3, elongated cells in both scabs terminating rows of suberised cells. In Plate XXII, fig. 4, elongated cells can be distinguished below the letter *C* and in the region of the letter *B*. Within the large cells of the infected areas there may be found bunches of the mycelium and dense groups of spores of the fungus which can be seen as dark masses at *F*.

The completeness of the suberised barrier extending beneath the infected area in Plate XXII, fig. 3, which is rather difficult to make out in the photograph owing to the section being unstained, would seem to indicate that extension of this scab is impossible. In other scabs distinct gaps of unsuberised cells can be found in some of the barriers and, as an abundance of mycelium is present in and around these unsuberised cells, it is probable that the organism passes through the barriers by way of such gaps. Also, amongst the cells of the barriers some are found to have thickened brown-tinted walls which give either no suberin reaction or a slight one, showing a thin deposit of suberin on their inner surfaces. These thickened cell walls (*E*) are illustrated more clearly in Text-fig. 1, where they extend, for the most part, radially from infected cells external to the barrier at *A* to the meristem at *D*. Further, in some instances isolated infected areas can be found beneath a completely suberised barrier, and the only evidence of the passing of the fungus from without to within the barrier is the presence of these chains of brown thick-walled cells, radiating from the infected area. In other instances, such as that portrayed in Text-fig. 2, the main route by which the fungus passes through the suberised barrier is very evident, but the only signs of its passage further inwards to the infected areas *E* and *E*, where *Actinomyces* threads were demonstrated, are the brown-tinted walls *B* leading to these areas. In Text-fig. 1 it will be noticed that fungal threads are present in some of the thick-walled cells, but they appear to be growing upon or within the remaining portions of the basal walls of these cells. This fact suggests that the fungus is able to travel through suberised barriers by growing within the cell walls. This it may easily do so long as the middle lamellae remain unsuberised, since culture solutions containing pectin as the only source of carbon can be shown to provide a suitable medium for its growth.

Text-fig. 1. Cross-section of a portion of the wound cork lining the margin of a moderate sized pitted scab. Drawn freehand from a magnification of 1080. *A*, collapsed cells. *B*, *Actinomyces* threads in basal wall (*C*) of cell. *C*, basal cell wall. *D*, wound cork meristem. *E*, thickened cell walls.

During the sectioning of the scabs of Plate XXII, figs. 1–4, and of many similar scabs, it has become evident that the course of development of a scab is as follows: the organism enters a young lenticel in which subsequently the meristem is stimulated and gives rise to radially elongated cells. Some of these become infected and eventually collapse and at the same time the collapsed cells become brown tinted, giving to

Text-fig. 2. Cross-section of a portion of a moderate-sized pitted scab showing the route by which *A. scabies* can penetrate beneath a suberised barrier. Drawn C.L. × 500. *A*, dark staining collapsed tissue. *B*, brown stained walls along which the fungus probably travels. *C*, shaded cells containing *Actinomyces* threads. *D*, cells with suberised walls. *E*, internal infected areas.

the young scab its characteristic colour. At no stage of their development can a suberin block be found in the tissues of young scabs. Further development of the scabs is due to tangential and radial divisions taking place in the lenticel meristem, thereby increasing the number of elongated cells. More of these cells become infected and subsequently collapse, thus widening the lenticel opening. After a time the meristem ceases to give rise to elongated cells and then produces a cork barrier about six

cells deep, the formation of which is not preceded by a blocking deposit. Penetration of this barrier by the fungus brings about infection of the tissues below and leads to the erection of another cork barrier some two to three cells deep, more or less cutting off the infected cells from the healthy tissue below. This barrier is probably laid down under the conditions set forth by Priestley and Woffenden (10) because it arises beneath a suberin block, the first formed wound cork. As a result of its appearance decay of the excised tissues follows and increases the size of the hollow or furrow of the scab, and any further extension of the scab that may occur is due to penetration of the successively exposed cork barriers and the erection of other barriers in the cortex beneath them.

The mode of entry of the causative organism into the potato tissues.

It was now thought advisable to test the behaviour of the fungus under experimental conditions towards (1) wound cork and the suberin deposits laid down upon the walls of cells beneath a cut tuber surface to ascertain whether the fungus could penetrate a suberised tissue such as the tuber periderm, and (2) the cutinised epidermis of the sprout.

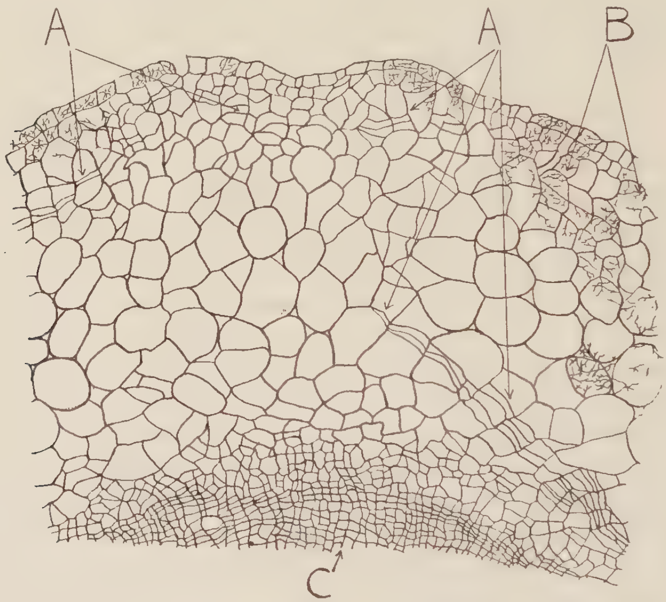
The periderm of tubers and the epidermis of sprouts are broken by lenticular openings, and the latter also contains stoma through which *Actinomyces* threads may find an easy entrance, but wound cork, such as is formed when a tuber is cut and allowed to heal under favourable conditions, contains no such openings. If then fungal threads are able to get through a protective barrier of this kind they must either pass through or between its cells. In the first experiment undertaken wound cork was obtained and the behaviour of the fungus towards it was tested in the following way: several Arran Chief tubers were sterilised in a 0·1 per cent. $HgCl_2$ solution and were then halved and left under sterile, moist, warm conditions for 18 days, until a complete healing of the cut surfaces had taken place. They were then inoculated with *A. scabies* from a culture grown in a Knops' glucose solution. A plentiful growth of mycelium occurred upon the cut surfaces, portions of which were removed and, after appropriate treatment, microtomed. An examination of the sections so made showed that a continuous suberised barrier of cork three to five cells deep, which stained well with Sudan III, was formed beneath the cut surface. Many of the injured cells external to the barrier were filled with fungal threads, but none was visible in the cells of the cork zone nor in the tissue below it. It is evident then that the fungus cannot penetrate the suberised cells of wound cork.

Paine (8) states that circular plugs, bored out of tubers, produce a layer of cork over their surface, and since such corky plugs would be very easy to handle for experiments of this kind, an attempt was made to obtain them. The variety of potato used was Great Scot, which produces wound cork easily, but although the plugs were kept under warm, moist, sterile conditions, the only protective layer that appeared on them was found to consist of a suberin deposit on the walls of the external cells. No true cork was produced. The experiment was thus a failure as regards its primary object. It proved useful, however, in another way, because some time after the plugs had been infected it was found that certain stages of the fungus had developed in them.

The action of the fungus upon the other protective tissue of the potato plant, namely the cutinised epidermis of the sprouts, was next investigated. For this purpose etiolated sprouts were used in preference to green sprouts or shoots, because, so far as is known, the fungus is only able to attack the underground parts of the plant. A number of such sprouts were grown under sterile conditions and inoculated. The first symptoms of infection were shown by the browning of the lenticels which proved to be due to the collapse of their outer proliferated cells. In the cells beneath this proliferated tissue fungal threads were found. In one instance a large drop of the inoculum was held between an etiolated sprout and its glass container, and at this spot a brown area developed upon the sprout. Sections of this area, which was free from lenticels, showed that many of the epidermal cells were filled with fungal threads, but it was evident, after treatment with Sudan III, that the cutin deposit on the outer walls of these cells was scanty, if present. Thus the fungus would have little difficulty in infecting the sprout, at such a point, by passing directly through the epidermal wall. Extensive invasion of the sprout tissues could also be seen which extended to the inner cortical layers (Text-fig. 3). Apparently the vascular elements were not affected, for a suberised barrier two to three cells deep had been erected beneath the invading fungus and this had effectively checked its inward progress.

The evidence that has been obtained so far from sectioning lenticels, and from the halved tuber and etiolated sprout experiments, indicates that the fungus can infect lenticels but is unable to penetrate through a normal barrier of wound cork. It may, however, infect the epidermal cells of an etiolated sprout when these cells are not protected by a well-developed cutin. Further support is thus given to the view, already expressed, that scab on the tuber originates by way of lenticel infection and not by direct infection of the periderm. Referring to the wound cork

of the sprout, no signs of any suberised cells other than the epidermal cells could be found external to these suberised barriers, and since the epidermal cells have already been stated to be scantily suberised, it is doubtful whether they could constitute a suberin block. Further, at the lower right-hand portion of the figure the suberised barrier of wound cork is deep seated while elsewhere it is almost of hypodermal origin, yet if the accumulation of fatty acids beneath the epidermis controls the point of origin of these wound cork areas, then they should all originate



Text-fig. 3. Transverse section of a portion of an etiolated, infected sprout. Drawn C.L. × 250. *A*, well-suberised cells formed beneath the infected areas. *B*, cells containing *Actinomyces* threads. *C*, vascular cylinder.

at approximately the same distance beneath the epidermis. That they do not do so but, instead, follow approximately the inner contours of the infected tissues, shows that it is the invading parasite rather than any suberin block which controls their point of origin. The close adjacency of the wound cork to the diseased area of the scabs of Plate XXII, figs. 3 and 4, lends support to this view.

If it is the invading parasite which controls the point of origin of the wound cork, then it is necessary, in view of the prevalence of wound cork in scab tissues, to consider in what way it may do so. Herklots (4) has shown that if the cut surfaces of potato tubers are exposed to a range of

artificially controlled hydrogen-ion concentrations, acidity encourages meristematic activity in the inner tissues of the tuber. Suberisation of the tissues produced by such a meristem is favoured by alkaline conditions. If then there is any similarity between the conditions under which the wound cork meristem of the shoot appeared and gave rise to tissues subsequently suberised, and those which Herklots found to favour similar processes in cut tubers, it is evident that the meristem of the wound cork of the shoot arose under acid conditions and its daughter cells became suberised under alkaline conditions. It is generally accepted that cell division occurs in a tissue along a gradient of hydrogen-ion concentration at the point where the principal protein of the tissue is at its isoelectric point. In the case of the potato the principal protein is tuberin with an isoelectric point of pH 4·4. Pearsall and Ewing (9), however, point out that potato wound cork cambium has a reaction of pH 4–5, and suggest that cell division can take place in the tuber at any of the points of minimal swelling of the tissue which they have found to occur at about pH 3·3, 4·5, 5·4 and 6·2. It would thus appear that while a wound cork cambium originates at about the isoelectric point of tuberin, cell division may occur in the tuber at points more acid or less acid than this point. In order to obtain a gradient of hydrogen-ion concentration including any one of these points within its range, it is evident that the gradient must become on occasion more alkaline than pH 6·2 or more acid than pH 3·3. Excepting the phloem, none of the tissues of the tuber is more alkaline than about pH 6·0, and the tissue where the wound cork of the scabs and the infected shoot arises is the cortex in which the pH is usually about 6·0. For cell division to occur in this tissue the gradient must include a portion less acid than pH 6·2. Obviously, in scabs and infected shoots, it is the invading *Actinomyces* which introduce this less acid portion of the gradient, since it does not occur naturally, and determine the contours of the wound cork. Proof of their ability to produce alkalinity in infected tissues is afforded by Sanford (11), who found that scabs of *A. scabies* when ground up with distilled water gave an extract with a pH of 7·2. The writer has also found that, in Waksman's (12) synthetic glycerin asparagin solution, both *A. scabies* Thaxter emend. M. and B., and *A. flavus* (M. and B.) gave rise to alkalinity over a wide range of initial pH.

Once cell division has occurred beneath the invaded areas of infected tuber, or shoot, fatty acids produced in the dividing cells pass into their walls and those of their daughter cells, and as the parasite approaches these cells, making conditions more alkaline in its neighbourhood, so

will these acids tend to become immobilised and give rise to a suberised tissue external to the wound cork meristem.

Previously it has been mentioned that no suberised barrier precedes meristematic activity in young pitted scabs. Such scabs develop in a young lenticel already possessed of dividing tissue at its base before infection occurs. After invasion, the parasite stimulates this tissue to division by setting up a gradient favourable to division over a wider region than existed previously; then as the infected cells collapse and the scab increases in size and the organism draws nearer to the lenticel meristem, the tissues immediately external to those in a state of division become suberised, giving rise to a wound cork in a manner similar to that described above. Incomplete suberisation of all the cells of the wound cork enables the organism to penetrate this barrier to its inward progress and to give rise, once or more often, to the sequence of events preceding the development of a wound cork.

Stages of the organism observed in potato cells.

In the infected sprouts, rootlets, and the portions of plugs immersed in water for a period, the fungus was present only as a vegetative, branching, septate, mycelium which, in the case of the plugs, was variable in thickness ranging from $0{\cdot}35$–$1\,\mu$.

In the portions of the plugs that had not been immersed in water for any length of time, and also in the tissues of mature scabs, sporogeneous hyphae were found. Their mode of branching and the characteristics of the spores they bore closely resembled those given by Millard and Burr (6) for *A. scabies* when grown upon culture media.

The structure of the tumulus type of scab.

The scabs of this type that have been examined were obtained by planting sterilised tubers in sterilised soil subsequently inoculated with *Actinomyces flavus* (Millard and Burr).

In appearance this type of scab is usually a slightly raised circular swelling on the tuber surface, with gently sloping sides leading up to a saucer-shaped central area. In most instances the swellings are isolated from one another, but sometimes two or three may join together to give a compound scab which differs from the isolated scabs in being larger and in having a slightly furrowed central portion formed by the union of the central depressions of its individual scabs. Such scabs differ from the pitted type in that the furrow is always very superficial, whereas the furrow of a pitted scab is deeper and is without raised margins.

The isolated tumulus scabs vary from 1 to 4 mm. in diameter, and the compound forms may be some 8 or 9 mm. broad.

In order to trace the development of this raised form of scab, scabs were microtomed at different stages of growth, including the earliest stage at which the causative organism could be found within their tissues and the stage at which the scabs had apparently ceased to enlarge.

An examination of the sectioned scabs showed that a smaller amount of the mycelia of the causative organism was present than was found in the pitted scabs, and, as a result of the scanty growth of *A. flavus*, it was impossible to find lenticels that appeared infected when viewed externally and in which the causative organism was present. Thus it is impossible to present direct evidence to show that the organism does infect lenticels, but as will be pointed out later, from indirect evidence, it is most probable that it does so.

The earliest stage of a tumulus scab in which the organism can be demonstrated is that given in Plate XXII, fig. 2, where there is depicted a scab which resembles a shallow pitted scab. The bulk of the tissue of the tumulus scab is produced by a wound cork meristem which gives rise, at first, to two or three layers of elongated, brown-walled, unsuberised cells, as at *A*, followed later by some twelve layers deep of regular well-suberised cells. If this type of scab has originated in a lenticel it is evident that all the characteristic lenticel tissue will have been obliterated by the activity of the wound cork meristem and the subsequent sloughing off of the tissues external to the wound cork.

The wound cork, it will be noted, forms a deep, continuous band beneath the scab and links up so completely with the periderm that only a slight trace of their union can be found at *B* and *B*. This line of junction may mark the exposed face of the periderm surrounding the lenticel in which the scab may have originated. No signs of either unsuberised cells or cells with radially thickened walls, such as are to be found in some of the pitted scabs, could be found in this wound cork, although wound cork cells with thickened radial walls were found in other of the tumulus scabs.

The causative organism is present in the cells around *A* in the form of short threads. Sporophores were absent from this stage of the scabs.

Some of the larger isolated scabs only differ from the one just described in that they are larger and their external cells contain a greater amount of *Actinomyces* threads.

In the remaining stage of the scab, namely the compound stage, there is no structural feature of note that distinguishes it from the earlier stages of the isolated scabs.

It has already been mentioned that in the earlier stages of the scab no sporophores of the fungus were present, but in the more mature scabs a few sporophores were found. They were mostly slightly branching with almost straight branches composed of barrel-shaped spores $1{\cdot}2\,\mu$ long by $0{\cdot}84\,\mu$ broad. Both the appearance of the sporophores and the size of the spores are in close agreement with the corresponding data given by Millard and Burr (6) for this particular fungus.

A comparison of the structure of pitted and tumulus scabs.

The chief distinction between the two types of scab is that the barrier of wound cork that is formed at the surface of a tumulus scab remains unpenetrated by the causative organism of the scab, whereas in the pitted scab this similarly situated barrier is frequently penetrated by the causative organism and is then succeeded by one or more wound cork barriers formed in the underlying tissue.

The reason for the difference in behaviour of the two fungi towards the wound cork of the infected tubers would appear to be that while *A. flavus* is unable to grow sufficiently rapidly within the tissues to prevent complete suberisation of the wound cork from taking place, *A. scabies* penetrates into the infected tuber comparatively rapidly, thereby preventing at times complete suberisation of the wound cork. Consequently the fungus is able to pass through the wound cork by way of any unsuberised cells that may be present in it.

A feature which has been already noted, which lends support to the view, previously expressed, that *A. flavus* does not grow so vigorously within an infected tuber as does *A. scabies*, is the comparatively small amount of mycelia of the former noted within the tumulus scabs. Excepting the differences in number of wound cork barriers and of the amount of fungal mycelia present, there is no other structural feature by which the two kinds of scab may be distinguished from one another.

Previously it has been shown that the production and suberisation of the first formed wound cork erected beneath pitted scabs is probably the result of alkaline conditions produced in the infected tissues. By growing the causative organism of the tumulus scabs, *A. flavus*, in a similar set of solutions to those in which *A. scabies* was grown, it has been found that the former is the more vigorous ammonia producer, giving rise finally to a greater degree of alkalinity than did *A. scabies*. If the behaviour of the fungus in a culture solution is any guide to its performance in infected tissues then *A. flavus* should bring about more rapid immobilisation of fatty acids and hence more rapid suberisation of

wound cork than does *A. scabies*. If this be so then the absence of unsuberised cells in the wound cork barriers of tumulus scabs and their presence in similar tissues of the pitted scabs may be accounted for on the assumption that better conditions for suberisation prevail in the tumulus scab.

It is rather surprising that in the tumulus scabs there is no sign of active cell division such as Millard and Beeley (7) showed to be characteristic of two raised types of scab on another host plant, namely the mound and knob scabs of mangels. These writers have shown that "pitted scab formation in the mangel is very similar to that of the ordinary type of common scab in potatoes," and it is natural to assume that a similarity must also exist between the structure of the raised types of scab on their respective hosts.

In order to find whether any such similarity did occur different kinds of raised scabs of the potato were sectioned and a parallel to the mound scab of the mangel was actually found in the scab of certain naturally infected tubers.

The structure of a naturally infected raised scab of the potato.

The scabs of this type that have been examined were taken from a tuber of unknown variety, the tuber being of ware size and abundantly covered with scabs which resemble those depicted by Millard and Burr (6) in Plate XVI, fig. 1 Bb. Many of the scabs are distinctly raised above the tuber surface and are usually isolated from one another, which suggests that the causative organism is unable to spread to any extent from the original point of infection. The diameter of the raised scabs may reach 6 mm., and the summit of the scab may be partly covered by tuber periderm and partly by wound cork, or it may be wholly covered by the latter. This apical area may be flat or wrinkled or it may contain a central hollow of variable extent. Other scabs upon this tuber are not raised above the tuber surface, but otherwise they appear to be identical in appearance with the raised scabs.

On microtoming the distinctly raised scabs it was found that in some of them, such as the scab illustrated in Plate XXIII, fig. 3, most of the raised portion consisted of closely packed thin-walled cells, often densely filled with protoplasm. Towards the exterior of the scab these closely packed cells are arranged in rows as at *B*, which terminate just beneath the wound cork meristem. Towards the interior of the scab these cells are less regularly arranged, but they show more distinctly than do the regularly arranged cells that this tissue has not been cut off from an

extensive wound cork meristem but is of cortical origin. External to this dividing tissue is an irregular band of wound cork which, although completely covering the dividing tissue in the figure as at *A*, in other scabs is sometimes broken by pressure from the extending cells below. In the figure there is no sign of any tissue external to the wound cork in the centre of the scab, but in other scabs where cell division has not occurred so extensively the centre of the scab usually consists of collapsed cells such as are found in pitted and tumulus scabs. Amongst this collapsed tissue are mycelia and sporophores of the fungus; the latter when examined carefully agreeing closely, in their method of branching and spore characteristics, with the data given by Millard and Burr (6) for *A. flavus*. The outstanding feature, however, of these distinctly raised naturally infected scabs is the central mass of dividing tissue capped by a wound cork, a condition which has its parallel in the mound type of scab described by Millard and Beeley (7).

In others of the naturally infected scabs, the raised portion is not so high as in the scab of the Plate XXIII, fig. 3, owing to either less cell division having occurred in them or to the more extensive invasion by *Actinomyces* mycelia that is evident and is accompanied by the subsequent collapse of the invaded tissue. Yet others of this type of scab are scarcely to be distinguished in structure from tumulus scabs or isolated pitted scabs in which only one wound cork barrier is present. These unraised, naturally infected scabs differ from the raised forms of the scab in that there is no sign of cell division beneath their wound cork barrier. They also differ in that the tissues external to the wound cork have not been obliterated in the former whereas they have disappeared in the latter. It is, however, very probable that the raised form of this scab has developed from the unraised form as the result of conditions developing beneath the wound cork barrier which have led to cell division occurring in this area independent of the wound cork meristem.

The similarity of the structure of the stages of the pitted and tumulus scabs, mentioned above, to the earlier stages of the naturally infected scabs suggests that in all of them a similar sequence of events must occur. This sequence, as has already been explained, commences with the invasion of a lenticel, continues with the development of conditions favouring cell division in the lenticel, and ends with suberisation of some of the cells cut off as a result of this division. How then does it come about that the raised kind of naturally infected scab differs from all the others that have been examined in that the sequence of events just outlined is followed by active cell division? Obviously the active cell division is

either a result of some characteristic that is peculiar to the organism causing the naturally infected scabs, or it automatically follows the sealing of the infected lenticel by a wound cork barrier. The first alternative is untenable because it has already been shown that cell division beneath the wound cork follows the formation of this tissue, by which time the parasite has been effectively isolated by the wound cork from the cells which subsequently divide, and in which the organism has not been found in any of the scabs examined. The second alternative appears to be more tenable than the first if it is remembered that when dormant tubers containing suberised lenticels are placed under conditions which stimulate respiration abundant cell division brings about rupture of the closing tissue of the lenticels. The tuber bearing the scabs examined had scarcely an uninfected lenticel on its surface, and consequently if it had not yet entered a period of dormancy, it seems likely that the cell division that has only occurred beneath some of the scabs is due to the same factors that bring about cell division in the lenticels of dormant tubers. A criticism may be levelled against this explanation to the effect that in many tubers affected with pitted scab all trace of lenticels may have been obliterated, and yet cell division, such as occurs beneath the raised scabs, is never found in these scabs in spite of the presence of one or more bands of wound cork beneath the scabs. Here, once a wound cork barrier of an infected lenticel has been erected within the tissues, this lenticel is not permanently closed, for, as has already been shown, *A. scabies* is able at times to prevent complete suberisation of the barriers and so permits the passage of air into the tuber. Also, beneath the first formed wound cork of the pitted scab, conditions favouring extensive cell division do not exist for any length of time, for fatty acids that may collect beneath the barrier become condensed into suberin as a result of conditions being made favourable to this process, by the organism, after it has grown through the barrier and invaded the tissues beneath.

If the literature on potato scab is now reviewed some interesting facts can be explained, providing, of course, we assume that the term "scab," as generally used, includes in all cases, at least, the "pitted" type of scab.

The period of susceptibility to scab.

Sanford (11) reports that "the critical period of susceptibility to scab infection occurs during the first ten days of tuber development," and Fellows (2) believes that "the period of susceptibility of a tuber may begin at any time in its growth providing it is forming an epidermis with

stomata and lenticels and that it ceases in growing tubers when the latter are no longer forming an epidermis with stomata or young lenticels."

Similarly, Wollenweber (13) has found that infection of mature tubers cannot be brought about unless the periderm is punctured artificially, whilst Fellows (2) and other workers have found it impossible to infect non-growing tubers by placing the fungal inoculum in contact with them. From the information that has been presented, regarding the mode of entry of *A. scabies* into tubers and etiolated sprouts, it would appear that potato tubers are only susceptible to this fungus up to the stage when their tissues are not *completely* protected by a well-suberised barrier.

Varietal resistance to potato scab.

Several workers (14–18) report that in field trials they have found varieties of potato which proved resistant to scab. In the case of *A. scabies* it is difficult to understand how a growing tuber, forming additional lenticels, can escape infection if it is exposed to the fungus.

Lutman (5), in examining the resistance of certain potato varieties to scab, came to the conclusion that "thickness of the skin determines the resistance of tubers to scab." He points out that the russet varieties which are moderately resistant have close textured lenticels partly buried under the skin surface and filled with small cells. So far as *A. scabies* is concerned, there would appear to be no reason why russet-skinned tubers should be resistant unless their deep set lenticels become suberised at a very early stage.

Effect of scab on growth of the potato plant.

In the potato-growing districts of N. America it has been recorded (1, 3) that reduction in the yield of tubers accompanies attacks of scab. Millard and Burr (6) have noted that brown, actinomycetous nodules develop upon the roots and stolons of infected plants, and the writer has produced similar nodules upon the roots developed in the etiolated sprout experiment. When the nodules were cross-sectioned they were found to be lateral rootlets that had been attacked and partly destroyed by the fungus as soon as they emerged from the parent root. In addition to the nodules, browned areas were also found upon the rootlets and were caused by the collapse and staining of some of the infected cortical cells. Beneath the infected areas cork cells were sometimes cut off by the pericycle, but they did not confine the fungus to the cortex, for it was found to penetrate into the vascular cylinder where it has brought about brown tinting and blackening of some wood vessels and of most of the

phloem cells. Fungal threads were clearly visible in these infected cells, and their presence in the conducting tissues most probably prevents the tubers of an infected plant from swelling, and may even curtail the number of young tubers that are laid down by the plant. The reduced yield, which is stated to occur in connection with attacks of common scab, is therefore readily accounted for if the "pitted" type of scab forms a fair proportion of the scabs reported upon and usually grouped together as common scab.

THE PATHOGENICITY OF *ACTINOMYCES* SPECIES TO POTATO TUBERS.

An attempt to test the pathogenicity of species of *Actinomyces* towards potato tubers by direct inoculation of etiolated sprouts of tubers proved impracticable owing to the necessity of keeping the tubers and sprouts in a moist atmosphere to enable *Actinomyces* in the inoculum to grow and infect the sprouts; in such an atmosphere unavoidable fungal contamination spreads from the tubers into the sprouts.

SUMMARY.

Pitted scabs. It has been shown that the pitted type of scab may originate in a tuber lenticel of which the meristem is then stimulated to division giving rise to an elongated type of cell. The newly divided cells are invaded by the parasite and subsequently collapse, meanwhile their contents and walls become brown tinted, giving to the scab its characteristic colour. As the destruction of the elongated cells proceeds the lenticel widens, and after a time its meristem becomes less active; its last-formed daughter cells cease to elongate and become suberised, forming a compact tissue, or barrier, of wound cork, cutting off the infected external cells from the healthy tissues below.

Further development is due to the organism being able to grow through incompletely suberised cells that are present in the wound cork barrier and infect the cells below. Here another wound cork arises, following closely the contours of its predecessor and permitting also the further inward penetration of the parasite, thereby enabling a third wound cork to develop beneath the scab. No more than three of these barriers have been found in one scab, but if active tuber growth could be prolonged it is probable that further barriers would develop there.

As a result of the development of the successive cork barriers an increasing amount of tuber tissue is excised and decays, leading to the extension of the scab which, in time, is found to be lined with the last formed cork barrier.

The parasite was observed within the cells of all stages of scab and

has been induced to grow within the living tissues of potato rootlets, etiolated potato shoots and plugs bored out of tubers. It has been observed to fructify freely in the tissues of mature scabs and infected plugs, where it displayed characters similar to those displayed when growing upon culture media.

The damage that the organism is capable of inflicting upon the root system of a potato plant is made evident.

The raised type of scab. The structure of two types of raised scab has been investigated. Both types have been shown to be more superficial than the pitted type and to possess only one wound cork barrier. The inability of their causative organisms to penetrate this barrier is suggested in explanation of their superficiality.

The two raised types have been found to differ from one another mainly in the restriction of abundant cell division, following the formation of a wound cork beneath the infected areas, to the naturally produced raised type.

The presence of *Actinomyces flavus* (Millard and Burr) was noted in artificially produced tumulus scabs where it displayed characteristics that have formerly only been observed on culture media. A species of *Actinomyces*, closely resembling *A. flavus* in sporophores and spore characteristics, was found in the naturally infected scabs.

An attempt has been made to explain the prevalence of wound cork beneath all the infected potato tissues examined, excepting those of young scabs, and its adjacency to these tissues on the basis of *p*H changes that the parasites may induce in the region of infection. It has been suggested that during the early stages of invasion the parasites may give rise to a gradient of *p*H favourable to cell division and extending from the infected to the healthy tissues. Later, owing to the increased activity of the parasite in the infected regions, conditions favouring suberisation are set up in the newly divided tissues leading to the formation of a wound cork barrier. Subsequent barriers will be formed in a similar manner below this first-formed barrier, following penetration by the parasite of the barrier preceding them.

It is pointed out that the period of susceptibility of potato tubers to, at least, the pitted form of common scab is probably determined by the degree of suberisation of the tuber lenticels.

The writer's thanks are due to Dr W. A. Millard, for helpful criticism and for supplying him with specimens of scabs and cultures of their causative organisms.

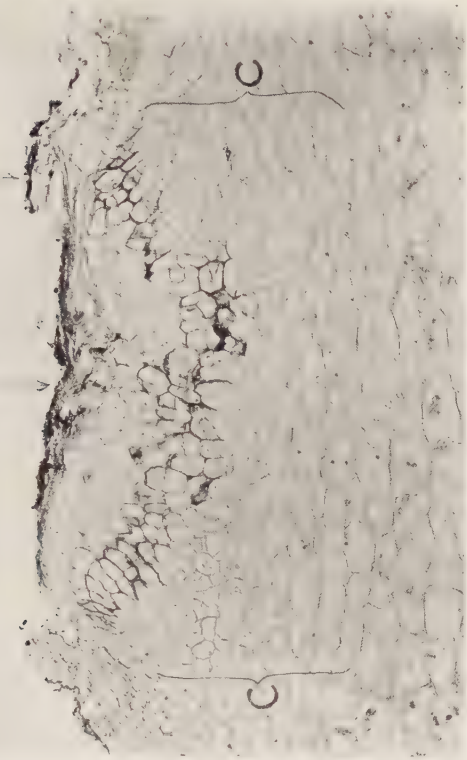
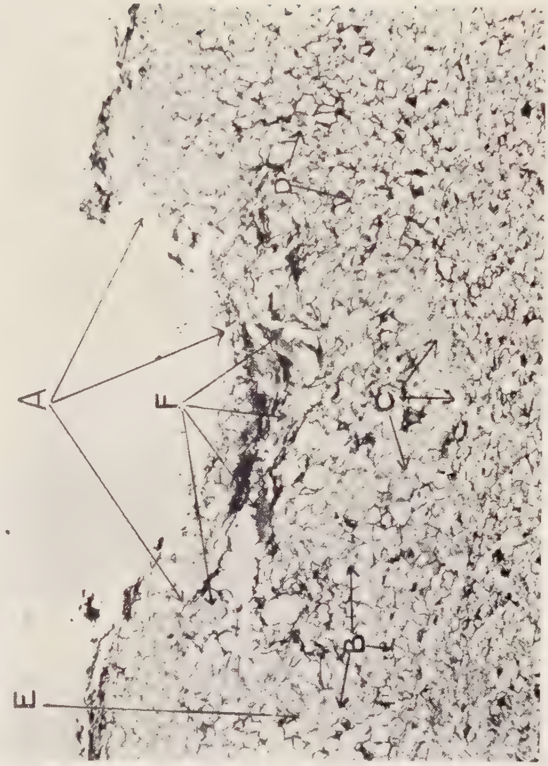


Fig. 2.



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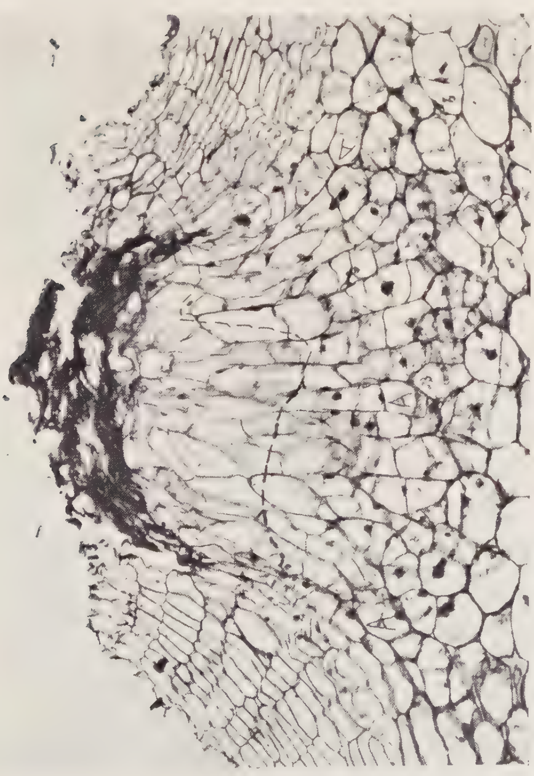

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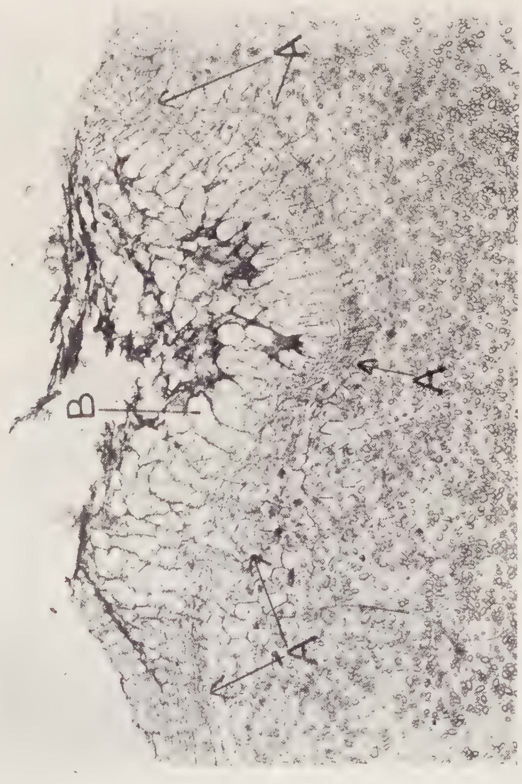
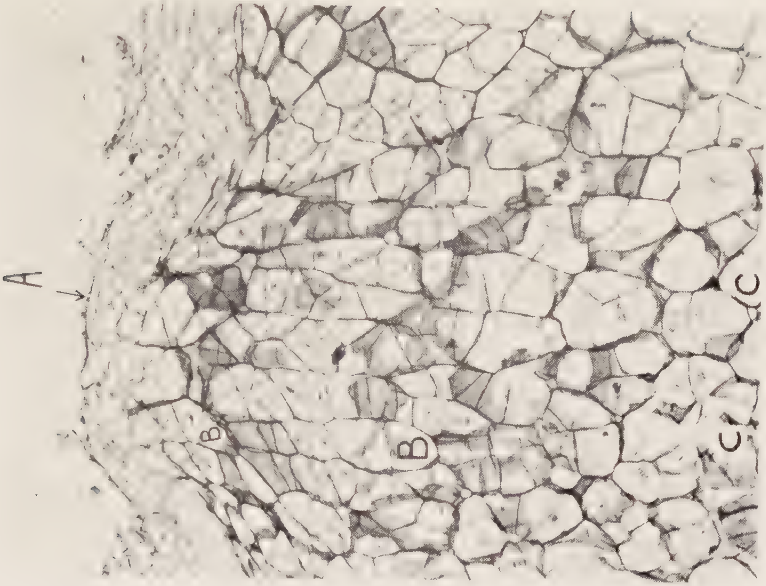


Fig. 3.

JONES.—The Histogeny of Potato Scab (pp. 313–333).



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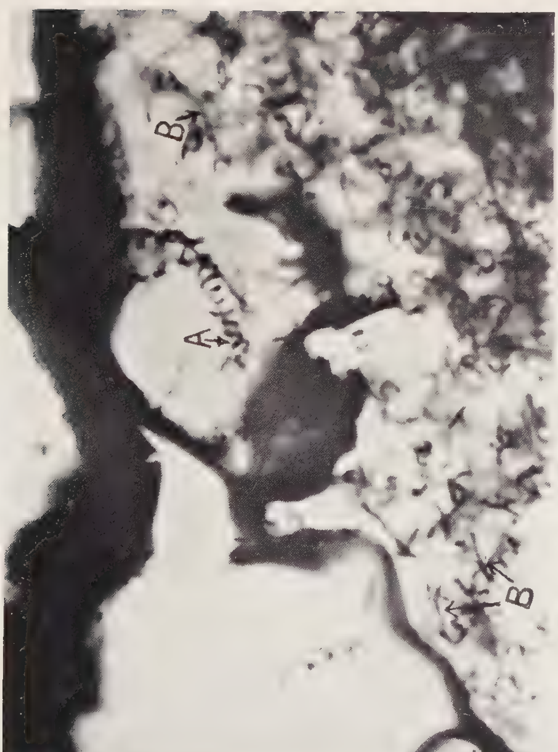

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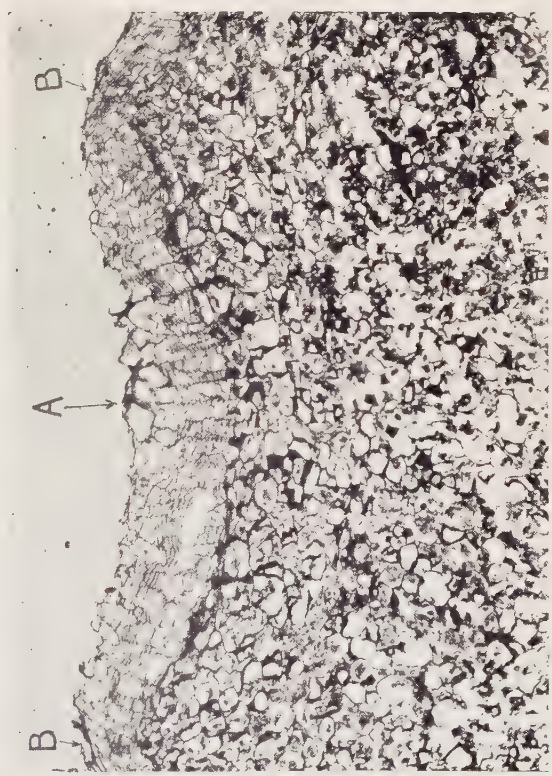

Fig. 2.

JONES.—The Histogeny of Potato Scab (pp. 313–333).

The writer's thanks are also due to Mr J. Manby for careful preparation of the photographs.

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EXPLANATION OF PLATES XXII—XXIII.

PLATE XXII.

Fig. 1. Transverse section of a very young scab (pitted type). 1 mm. in diameter. ×100. Stain. Delafield's haematoxylin. *A—A*, lenticel meristem. All cells external to the dotted line contain *Actinomyces* threads.

Fig. 2. Transverse section of an older scab (pitted type) than that of Fig. 1. ×45. Stain. Light green and safranin. *A*, junction of the collapsed tissue and the tuber periderm. *B*, collapsed tissue. *C*, starch-free tissue.

Fig. 3. Transverse section of a mature isolated scab (pitted type). ×30. Unstained preparation. *A*, wound cork meristem.

Fig. 4. Transverse section of a later stage of scab (pitted type) than that shown in Fig. 3. ×22. Stain. Delafield's haematoxylin. *A*, the first protective barrier of cork. *B, C, D*, centres of invasion of the fungus after it has penetrated the barrier at *A—A*. *E*, cork cells produced by a dividing cortical cell. *F*, masses of fungal spores and mycelia.

PLATE XXIII.

Fig. 1. Transverse section of an externally placed cell of a mature scab (pitted type). ×1080. *A*, sporogenous coil with at least eight turns in its spiral. *B*, filaments with dark staining contents.

Fig. 2. Transverse section of a young, artificially produced tumulus scab. ×33. *A*, brown-walled cells. *B*, probable junction of periderm and wound cork.

Fig. 3. Transverse section of a portion of a naturally infected raised scab. ×80. *A*, wound cork. *B, B*, a chain of cells. *C, C*, starch-containing cortical cells.

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A BOTANICAL STUDY OF PASTURE FORMATION

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(With 5 Graphs.)

CONTENTS.

INTRODUCTION.

THE present study concerns the field known as Long Balls on the Seale-Hayne Agricultural College Farm, Devonshire, and was carried out while the author was on the staff of the College. The analyses and notes were made during the period of the experiment, but no time was available to deal with the results till quite recently.

The experiment was laid down to test the stock-carrying capacity of five seeds mixtures similar to certain pastures which were in the county. The head of stock on each of the plots (each $2\frac{1}{2}$ acres) was increased, reduced, or removed from time to time according to the growth of the vegetation. This afforded an opportunity to watch and record the various changes in the flora under a careful system of grazing.

FIELD OF LONG BALLS.

The soil is a medium loam lying on the Upper Devonian system at an elevation of 350 feet. The underlying rock is a shale (locally known as shillet), while the soil is silty, tending to become sticky when moist. The previous cropping of the field had been: 1917, roots; 1918, barley; 1919, seeds; 1920, seeds; 1921, roots; 1922, roots; 1923, wheat; 1924, oats. The

seeds mixtures were sown down with the oat crop (April 11th, 1924). The manuring was 4 cwt. kainit, 3 cwt. superphosphate, and 1 cwt. nitrate of soda per acre. In 1919 and 1920 there was a heavy infestation of Wild Carrot (*Daucus Carota*). The seed of Wild Carrot was scattered, survived, and appeared in some of the grass plots. Further details, such as cost, etc. of seeds mixtures, will be found in the *Guide to Field Experiments and Plots*, 1924 (11).

The experiment.

The seeds mixtures sown were:

	Plots I lb.	II lb.	III lb.	IV lb.	V lb.	
Perennial Rye Grass (Irish) ...	8	16	16	16	12	*Lolium perenne*
Italian Rye Grass	8	—	—	—	4	*L. Italicum*
Cocksfoot (New Zealand)	10	10	10	10	6	*Dactylis glomerata*
Timothy (Scotch)	4	4	4	4	—	*Phleum pratense*
Rough-Stalked Meadow Grass ...	—	—	—	—	1	*Poa trivialis*
Late-flowering Red Clover (Suffolk)	4	4	4	—	—	*Trifolium pratense*
Cornish Marl (L.R.F.C.)	—	—	—	—	5	*T. pratense*
Broad-leaved Red Clover	—	—	—	4	—	*T. pratense*
Black Medick	1	1	1	1	—	*Medicago lupulina*
Wild White Clover (Kent) ...	1	—	1	1	1	*Trifolium repens*
White Clover	—	1	—	—	½	*T. repens*

After the removal of the oat crop (1924) there was a heavy aftermath consisting chiefly of Red Clover. In Plot IV the Broad Red Clover "went down," and in 1925 there was still some evidence of this. All the plots were lightly grazed with sheep after removal of the corn crop. During the winter all were dressed with 8 cwt. of 40 per cent. basic slag and 1 cwt. of muriate of potash. All plots were stocked by April 28th, 1926, but Plots I and V had to be stocked previously owing to their earlier growth.

The rainfall is plentiful but, in spite of this, summer drought is the rule rather than the exception. Another difficulty is that there is a very marked flush of spring growth and generally a less marked one in autumn. This often renders the stocking of pastures rather difficult not only in the pasture fluctuation but also in the difficulty of adequate water supply. The annual rainfall for the years during which the experiment was running was: 1924, 47·77; 1925, 39·96; 1926, 33·37; 1927, 39·22 inches.

Method of analyses.

For investigating the nature of the pasture in the five plots the percentage area of ground covered by each species was measured by a wire grid 10 in. × 10 in., the method being similar to that adopted by

Armstrong (1). The grid method was selected as it did not in any way interfere with the plots and, moreover, made comparison possible with other Devonshire grasslands (3). For analyses of the hay sub-plots, a large sample was drawn and the material air dried. After separation into species each was weighed and the percentage thus obtained.

Apart from these figures careful notes were made of the state of the vegetation both at the dates of analyses and at intermediate periods. The information so obtained supplemented the analyses and took greater note of occasional weeds and other plants of significance.

Species which made any serious contribution to the herbage as measured by percentage ground covered were arranged in graph form, representing the percentage ground covered against the time of the year. This method was adopted as affording a better "bird's eye view" of the whole year's vegetative growth and fluctuations than a series of tables. The results of the hay sub-plots are left as weights, but the total annual yields of all plots are represented by a diagram to illustrate the species which contributed the bulk of the vegetation for each of the three years during which the experiment was conducted.

The experiment on the agricultural side was judged by the number of days' grazing obtained per plot; that is, the number of days and the head of stock carried each day: bullocks, sheep, and lambs. The method is open to criticism, since it takes no detailed account of the condition of the stock or the gain or loss in weight or the prices which the animals would command in the market. In the early stages Plot I had most keep and carried most stock. Plot V ranked next. After a time differences in the plots became much less evident, with the exception of No. II which deteriorated. We may add that in several examples tested in the county we found our placing of plots by botanical analysis and notes to be in the same order as reckoned by the farmer estimated on the selection and grazing of the stock. This relationship between botanical composition and yielding capacity of grasslands has just lately been pointed out by Stapledon and Thomas (17).

A point of considerable importance is that it will take fewer head of stock to graze down a plot which is palatable than what will be required to graze down a plot which is not so relished. On this account the botanical records with the condition and rapidity of growth of the various plots is probably more reliable than the stocking capacity, particularly where this is not supplemented by the gain or loss in weight of the stock.

TABLE OF ANALYSIS.

Hay sub-plots, 1925–7.

Figures indicate the percentage weight of each species present in the hay.

	1925										1926															1927					
	May					July					April					May					June					June					
Plots ...	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	
White Clover	9	9	3	2	1	3	13	1	2	3	13	4	1	1	5	13	1	1	19	4	6	.	1	11	10	4	.	1	5	3	*Trifolium repens*
Red Clover	1	17	24	17	24	5	28	50	14	24	.	.	.	5	3	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	*T. pratense*
Alsike Clover	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*T. hybridum*
Black Medick	5	2	13	21	.	1	7	7	17	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	*Medicago lupulina*
Perennial Rye	15	56	47	54	46	20	37	29	51	35	30	46	75	62	54	49	86	96	52	61	34	3	32	34	30	4	2	15	19	10	*Lolium perenne*
Italian Rye	60	1	1	.	20	64	2	.	.	21	17	.	.	.	.	19	T.	.	.	.	15	3	.	2	10	3	1	.	.	1	*L. Italicum*
Cocksfoot	3	3	4	2	7	1	5	.	5	4	30	47	13	23	35	11	10	2	26	12	32	33	53	34	22	67	75	72	54	69	*Dactylis glomerata*
Timothy	4	3	5	2	T.	4	3	3	4	T.	6	.	7	5	T.	8	T.	1	3	T.	9	9	6	13	T.	10	10	6	6	T.	*Phleum pratense*
R.-S. Meadow Grass	1	4	1	1	T.	.	.	1	3	12	2	2	3	.	3	.	2	.	.	23	.	.	.	.	24	.	1	1	5	12	*Poa trivialis*
Yorkshire Fog	1	1	2	1	.	.	4	5	2	1	1	1	1	4	.	.	1	.	.	.	2	.	5	3	.	6	2	3	1	1	*Holcus lanatus*
Bent	.	1	.	.	1	2	.	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T.	T.	T.	T.	T.	*Agrostis palustris* (Huds.)
Soft Brome	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	1	.	2	.	*Bromus mollis*
Weeds and miscellaneous plants	1	2	.		1	.	1	2	.	.	1	.	.	.	.	.	.	.	.	.	2	2	3	3	3	4	8	2	6	4	Weeds and miscellaneous plants

T. = trace

Pasture plots, 1925.

During the first year, the graphs gave a good bird's-eye view of the fluctuations of the chief species in the plots and the process of settling down. In all plots except No. II Perennial Rye Grass and Wild White Clover dominated the vegetation, with Cocksfoot a good second. Red Clover, which was present in quantity at the beginning of the year, had virtually disappeared by autumn. This was not due to competition with Rye Grasses and Cocksfoot so much as the selective grazing of sheep. The sheep after the early growth neglected Rye Grasses for Red Clover and, but for cattle grazing, Rye Grasses would have been left to flower and fruit, completely upsetting the pasture.

Summer drought affected all plots except No. I, but No. II suffered most, having White Dutch in place of Wild White Clover. The presence of Rough-Stalked Meadow Grass did much to assist No. V in maintaining a good bottom turf. The plots were remarkably free from any quantity of weeds, No. II, as might be expected, suffering most and being the only plot with Wild Carrot (*Daucus Carota*). The other weeds present were *Cnicus arvensis*, *Geranium molle*, *Agrostis palustris*, *Holcus lanatus*, *Cerastium arvense*, *Convolvulus arvensis* and an occasional *Rumex* spp. *Cynosurus cristatus*, *Poa annua* and *Phleum pratense* were present but not in sufficient quantity to be represented on a graph.

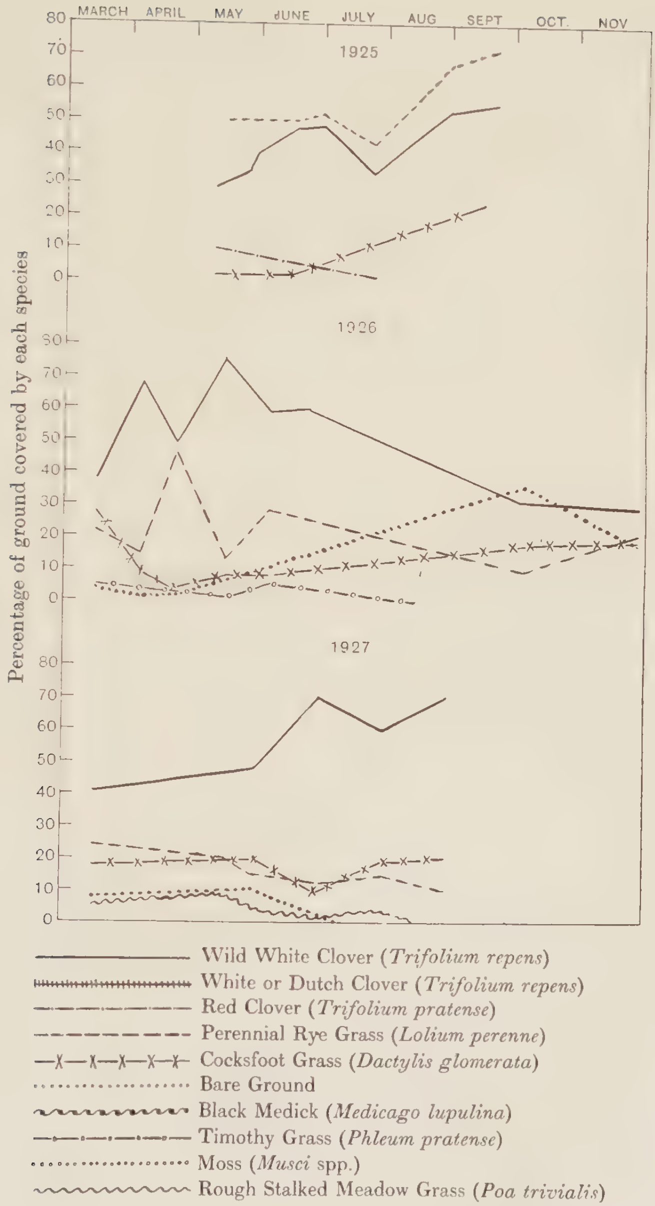
Hay sub-plots, 1925.

The small square areas fenced off for hay were analysed, so as to indicate the nature of the hay cut obtained from the pasture. No real comparison is possible, and as stock broke into two of the plots, any attempt at comparison of weights between the hay sub-plots is out of the question.

Rye Grasses and Red Clover supply the bulk of the hay, while Black Medick (*Medicago lupulina*) in Plots III and IV adds its quota, as the diagram for 1925 shows. Italian and Perennial Rye Grasses in Plot I completely suppressed Red Clover.

Pasture plots, 1926.

The graphs for 1926 reveal the fact that except Plot II, all the plots show a very strong resemblance. This rapid settling down to a homogeneous vegetation was the feature of the vegetation changes. There were small differences and fluctuations, but they were due to slightly different stocking and the initial difference of the seeds mixtures.



Plot I. Graphs illustrating seasonal fluctuation of species.

There was a severe drought which affected all plots, but its effects had almost gone by the end of the season, in spite of the fact that in Plot II nearly 60 per cent. of the ground had no vegetation in the early days of October. The two other points of interest are the increase of Cocksfoot (particularly due to its drought resistance) which equals and finally surpasses Rye Grass, and the fact that the plots show practically the same vegetation at the end of the season as at the beginning: clear evidence that the pasture in the plots has virtually settled down to a definite vegetation.

Weeds were as in 1928, and considering the long drought could not be called plentiful. Plot II had a large proportion of *Poa annua*. Timothy made its first appearance in quantity in Plots I and II.

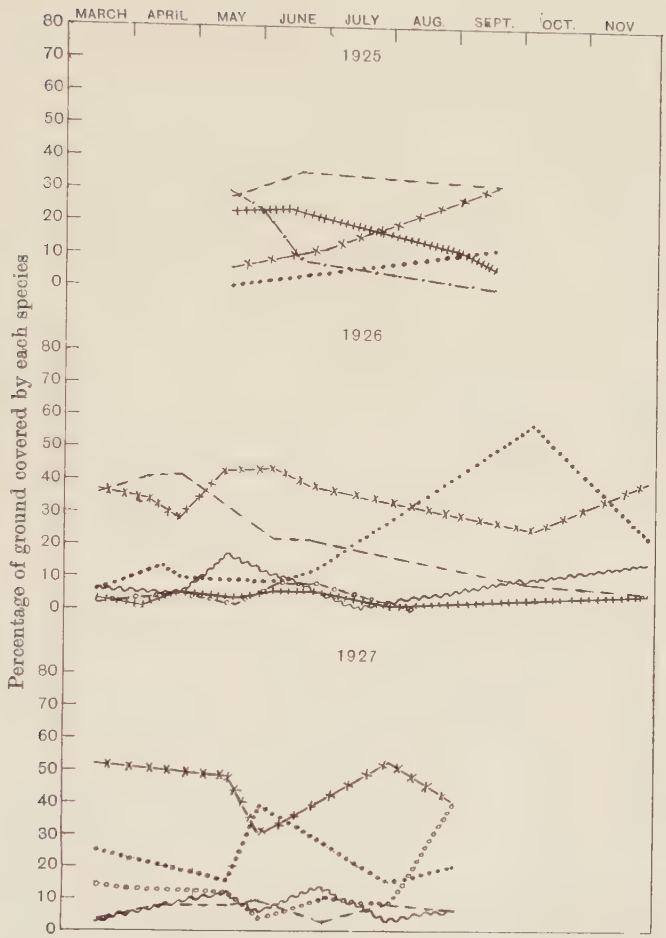
Hay sub-plots, 1926.

The hay sub-plots, as the analysis indicates, show considerable resemblance; for Perennial Rye and Cocksfoot gave the bulk, while Timothy, Wild White Clover, and in Sub-plot V Rough-Stalked Meadow Grass, gave a reasonable proportion. The diagram shows that Cocksfoot ranked next to Perennial Rye in yield. Weeds showed a tendency to increase. The low figures for Cocksfoot in the first two cuts in Sub-plot III were due to cattle breaking in and grazing. The Perennial Rye recovered more rapidly and in consequence held an initial advantage (17). The quantity of Rough-Stalked Meadow Grass in Sub-plot V adversely affected Cocksfoot.

Pasture plots, 1927.

The graphs (1927) show that Plots I and V were marked by remarkable similarity, as were Plots III and IV. The chief differences between I and V were a marked peak in Wild White Clover at the end of June in Plot V and no bare spaces. Plot II as before was quite exceptional with Cocksfoot completely dominant, bare space and moss ranking next for area of ground covered. In all plots except V there was much Annual Meadow Grass mixed with the Rough-Stalked Meadow Grass. It is evident that the similarity of I to V and III to IV is due to the presence of Italian Rye in the first two and none in the last two. Even so, the difference between the two groups is not marked and in course of time would completely disappear under similar treatment. It shows the vital importance of the skilled management of pastures, especially on the grazing side.

Weeds did not occupy much space and were not so numerous as in previous years, except in Plot II.



White or Dutch Clover (*Trifolium repens*)
Red Clover (*Trifolium pratense*)
Perennial Rye Grass (*Lolium perenne*)
Cocksfoot Grass (*Dactylis glomerata*)
Bare Ground
Black Medick (*Medicago lupulina*)
Timothy Grass (*Phleum pratense*)
Moss (*Musci* spp.)
Rough Stalked Meadow Grass (*Poa trivialis*)

Plot. II. Graphs illustrating seasonal fluctuation of species.

Hay sub-plots, 1927.

In 1927 only one cut was obtained and analysed, as the drought prevented any adequate growth till very late in the season, and by that time the writer had left the area. Cocksfoot had now completely dominated all the sub-plots. Rye, except in Sub-plots III, IV and V, had fallen to a low figure, while Timothy in most cases showed an increase. Italian Rye was still present, but only in small traces. Wild White Clover was present in all sub-plots except No. II, and gave a surprising proportion considering the tall vegetation against which it had to compete (15).

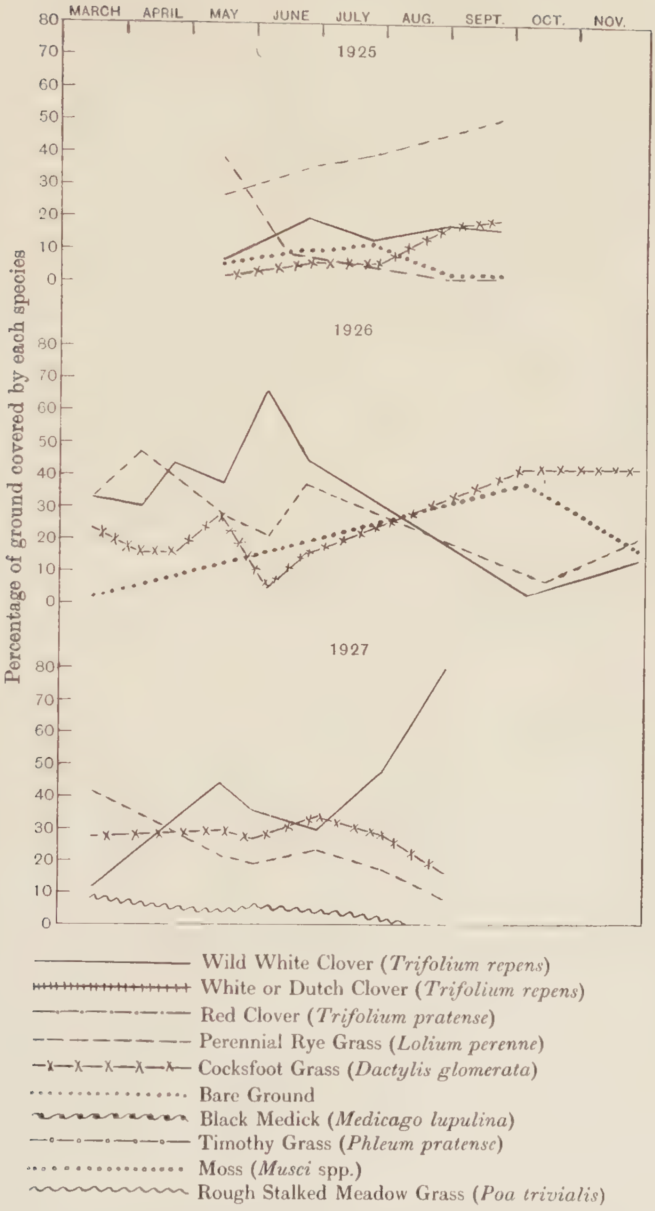
The condition of the hay sub-plots in 1927 is not comparable with that of the demonstration plots (5). There had been no manures added to the soil of the demonstration plots for several years, while the soil of Long Balls was adequately manured. This is evident from the comparatively small proportion of weeds present in the hay sub-plots of Long Balls.

Discussion.

The small hay sub-plots (page 337) were important, as they afforded an opportunity of comparing the vegetation of hay and pasture from the same seeds mixture. No close comparison is possible, since pasture was judged by the percentage ground covered by the chief species, while hay was calculated by weight. By 1927, the hay sub-plots had 30–40 per cent. bare ground, a figure only equalled in pasture by Plot II.

The pasture plots were of great interest because they were of such a size that they were treated as small fields and consequently they had an important agricultural and economic interest apart from botanical considerations. Further, the fact that the treatment and mixed grazing were at all times carefully watched and controlled, adds greatly to the value of the experiment and to the result. Under these practically ideal circumstances the botanical composition of the pastures, their changes and seasonal fluctuations are valuable since they provide an almost standard result for comparison with other cases under comparable conditions. The plots show how quickly and effectively good pasture can be achieved, and—as significant—the paramount importance of sound and mixed grazing (9).

The graphs of 1926 and 1927 show that, as judged by the percentage ground covered, there are generally two characteristic peaks in the growth of Wild White Clover and Perennial Rye Grass (17). This we have noted as very characteristic of many good pastures in Devonshire. The

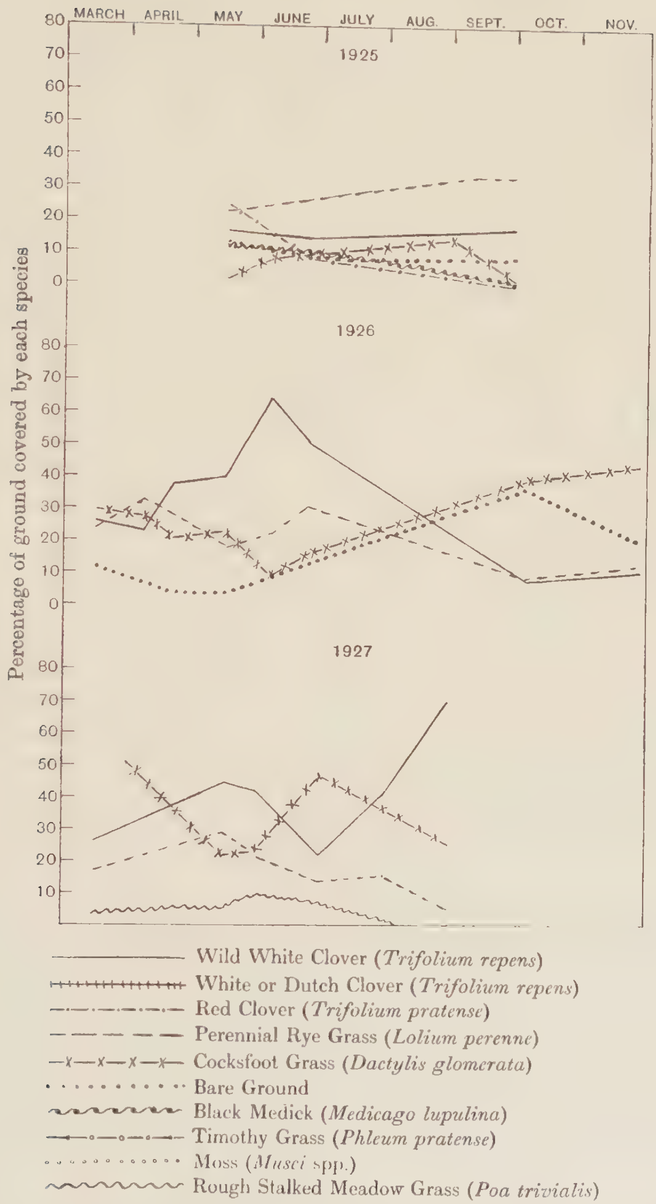


Plot III. Graphs illustrating seasonal fluctuation of species.

peak of Wild White Clover is usually accompanied by a fall or a depression of Perennial Rye Grass and *vice versa.* When these two are not the chief species, another species such as Cocksfoot may alter their inter-specific alternation and either replace one of the species or modify the usual fluctuation. In the pasture plots Cocksfoot was the only species to change the alternation between Wild White Clover and Perennial Rye Grass. Plots III, IV and V all illustrate this interference by Cocksfoot towards the end of 1926.

The most noticeable feature in the early stages of pasture formation was the rapid decline and disappearance of Red Clover, and the much earlier growth of plots containing Italian Rye Grass (I and V). Another factor which affected all plots was that, after the early spring growth of Rye Grasses were eaten down, sheep grazed Red Clovers very hard, neglecting the Rye Grasses, and this greatly increased the depressing effect of the Rye Grasses on Red Clover (14). Had cattle not grazed down the Rye Grasses, an almost hay-like condition of the pastures would have developed. This hay-like growth of Rye Grasses is frequently found in good pastures where grazing is chiefly confined to sheep. Where there are no cattle available to keep these stems down, a mower would correct this injurious hay-like condition and greatly improve the turf, as has been pointed out by Stapledon and Jones (14).

The general progress of the plots, as the graphs indicate (1926), was toward a similarity of flora, Plot II being the only exception. The progress of Wild White Clover was remarkable, for even in the hay sub-plots (page 337) it gave a high yield. As Red Clover had not persisted (8), Cocksfoot in both pasture and hay showed a marked increase, only Plot V with the combination of Wild White Clover, Perennial Rye and Rough-Stalked Meadow Grass showed Cocksfoot held in check. It is evident from this that Rough-Stalked Meadow Grass must be sown (15). The fact that the composition of the plots was similar at the beginning of 1926 and at the end of the season is significant, indicating that the pasture had virtually settled down to a fixed flora. Moreover, the similarity of flora of the various plots by 1927, in spite of initial differences of seeding (*e.g.* 8 lb. Perennial Rye Grass in Plot I and 6 lb. Cocksfoot in Plot V), shows that when circumstances are favourable to a species it will increase from a light seeding till it occupies a large proportion of the ground. In fact, the proportions of the more important species will fluctuate from an initial position of mobile equilibrium till a more fixed proportion is reached which is in equilibrium with the factors affecting the growth and spread of the various species.



Plot. IV. Graphs illustrating seasonal fluctuation of species.

The proportion of Rye Grasses in I was about 50 per cent. of each at the beginning of 1925, but at the end of the year Italian Rye was contributing only 25 per cent. of the total Rye Grass yield. In 1926 Italian Rye fluctuated from 10 to 15 per cent. and to 5 per cent. of the total Rye yield in 1927. The steady yield of Rough-Stalked Meadow Grass in Plot V was important, as it did much to assist in the formation of a good bottom to the pasture (13).

The fact that II and IV suffered very severely from drought is of significance, since II had Dutch White (13) and IV had, in the early stages, failed to develop such a good carpet of Wild White Clover as I, III and V. It emphasises the vital necessity of a good growth of Wild White Clover as a safeguard against summer drought (12).

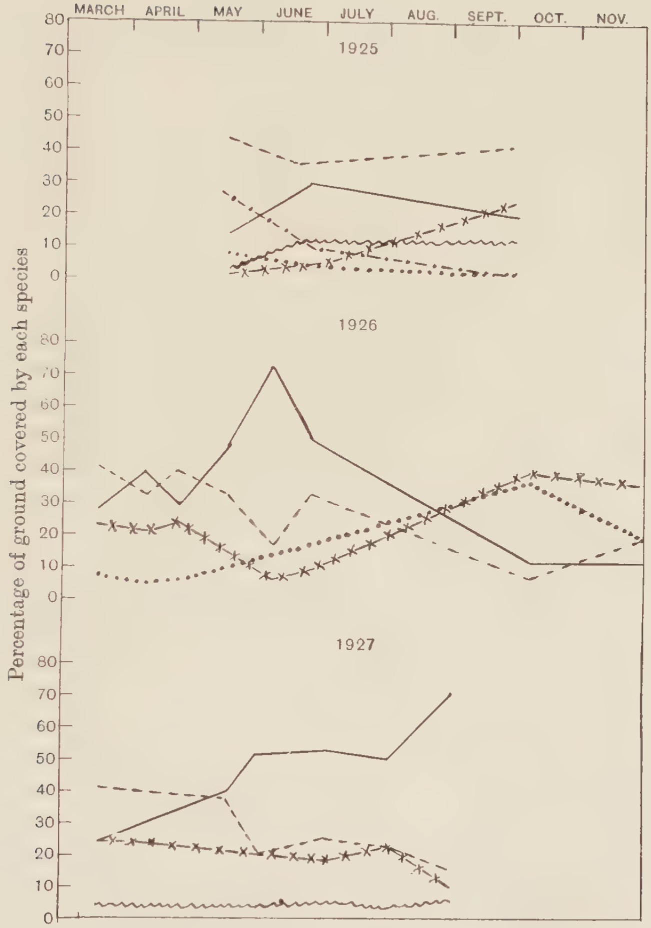
The failure of pasture with no Wild White Clover has been shown in Wales, while the physiological effect of drought and lack of nitrogen (13) affecting the health of certain grasses has been dealt with elsewhere (4). The importance of Wild White Clover in pasture formation is clearly demonstrated, as it was by Gilchrist many years ago (7).

The remarkable botanical similarity of all the plots (except No. II) is in agreement with the similarity in flora of the demonstration pasture plots (6). There is a further resemblance between the flora of the present pasture plots and that of the demonstration pasture plots previously described (6). It is a point of considerable practical importance since it demonstrates that with care valuable botanical information may be obtained with small-sized plots, thus avoiding the necessity of large and costly experimental plots. Not only so, but in attempting to approach the "practical" the results may at times be misleading, as Stapledon and Davies have very recently stated (16).

The graphs for the pasture plots 1925, 1926 and 1927 show very clearly the competition between Cocksfoot and Perennial Rye Grass (15). The important part played by Wild White Clover in association with Perennial Rye Grass is also evident if Plot II is compared with the others.

Timothy was not a success. This was due to the hard grazing of sheep and also to the severe droughts in summer. The hay sub-plots (5) clearly indicate that it is far more successful in hay than in pasture. This is in keeping with the findings of the demonstration plots (5, 6). In many pastures in South Devon Timothy is not successful, and might safely be omitted from the seeds mixtures.

The instance of Cocksfoot giving less weight in hay after Sub-plot III was broken into and grazed by stock is of importance. It is obvious from this that Cocksfoot recovers from grazing at a slower rate than Rye



Wild White Clover (*Trifolium repens*)
White or Dutch Clover (*Trifolium repens*)
Red Clover (*Trifolium pratense*)
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Timothy Grass (*Phleum pratense*)
Moss (*Musci* spp.)
Rough Stalked Meadow Grass (*Poa trivialis*)

Plot V. Graphs illustrating seasonal fluctuation of species.

Grass (9). It also explains why of two similar mixtures the hay frequently shows far more Cocksfoot present than the pasture. This difference is still more accentuated when cutting alone (for hay) is compared with grazing alone.

A further point of considerable practical importance was pointed out by Mr A. Blenkinsop, B.Sc., Soil Chemist at Seale-Hayne Agricultural College. While discussing with him the effect of the lack of Wild White Clover on the health of the pasture in Plot II, he pointed out that on analysing the soil Plot II was not only much more deficient in available nitrogen than the others but that the acidity as measured by the pH was lower. The figures (pH) which he has kindly given me are for Plots I to V: 7·12, 6·61, 7·26, 7·18 and 7·41 respectively. As the plots were part of one field which was fairly homogeneous, one is forced to the conclusion that the quality and nature of the vegetation has a marked influence on the pH of the soil. For woodland soils this feature has been described by Salisbury (10). That a definite change in acidity may become evident in such a short time in agricultural land is a matter of more than academic interest and may explain why the vegetation of certain grasslands retrogresses at such a rapid rate. If this is of general occurrence, then in semi-natural and natural grasslands the effect of the vegetation on the soil must be still more marked and may explain the difficulty of successfully improving some types of grassland. Probably the high percentage of *Musci* spp. in Plot II did much to promote acidity.

Another interesting point is the fact that in the hay sub-plots *Agrostis* did not make much progress. In the pasture plots even in No. II *Agrostis* never appeared in any quantity. The chief reason for this was largely the dry periods in summer preventing it making any real initial progress. The constant treading of cattle and the close grazing also helped to prevent *Agrostis palustris* making any progress: a point recently noted by Bates (2). Once established, *Agrostis* is capable of withstanding a very fierce drought, and under such conditions may outlast Rye and rival even Cocksfoot. In hay, so long as there is a good top canopy (while fertility is maintained) *Agrostis* makes little progress, but once that fails its growth and spread may be rapid. We have seen instances where *Agrostis* has been held in check in pastures by taking an occasional hay crop in the second half of the year, and thus shading out the *Agrostis* which is more prostrate. In an example we examined in Devonshire, *Agrostis* was greatly diminished and Perennial Rye Grass much increased by this method.

It was obvious from watching the stock grazing that if the plots had been grazed exclusively with either cattle or sheep, the vegetation would

have neither developed so good a turf nor settled so quickly into so stable a pasture. The benefit of the mixed grazing was that the one type of stock counteracted the defects of the other, thus preventing any single species of plant being unduly favoured. This was much more evident in the earlier than in the later stages and indicates the vital importance of judicious stocking in the formation and successful management of a pasture. Other things being equal, this biotic factor is one of the most important, if not the most important, in the formation and successful continuance of a good pasture. This has just recently been noted by Troup and Williamson (18).

There is perhaps one question in conclusion which arises from the present investigation. Since Red Clover in quantity depresses Wild White Clover and Rye Grasses, should Red Clover be used in seeds mixtures for permanent or long ley pastures? If included, should the quantity be reduced to less than 4 lb. per acre? Since Red Clover is chiefly valuable for adding to the hay cut usually taken before grazing commences, might it not be better to speed up pasture formation by omitting Red Clover and sacrificing the heavier hay cut? We have previously pointed out the fallacy of sacrificing the subsequent pasture by sowing a "hay" seeds mixture for heavy hay cuts during the early stages of growth (6). It seems that, except where the necessity of circumstances demands hay, it would be better to sacrifice the hay cut for speedier and better pasture formation. Where pastures are to remain down for a number of years, the much more rapid formation of a good turf and ability to withstand drought easily outweighs the value of a hay cut. It is certainly a question that deserves attention and a thorough trial. The recent publication of the *Welsh Plant Breeding Station*, Series H, No. 11, bears out this point where simple mixtures with no Red Clover were very successful in turf formation (17).

SUMMARY.

Where summer drought is liable to occur it is impossible to form a satisfactory turf in pasture without Wild White Clover. White or Dutch Clover cannot replace Wild White Clover except during the first year of grazing.

Italian Rye Grass is of considerable value, not merely on account of its early growth, the shelter afforded slower-growing species and helping to conserve surface moisture, but it greatly assists Wild White Clover by competing against Red Clover.

The two most successful plots were those with Italian Rye Grass in the seeds mixture.

The addition of Rough-Stalked Meadow Grass was a distinct success and it should be included in seeds mixtures for long leys or permanent pastures in South Devon.

With sound mixed grazing, all the plots—except No. II—gradually developed a flora of very similar proportions, in spite of initial differences.

The chief feature of the best plots was the Wild White Clover—Perennial Rye Grass association plus Cocksfoot, and in one case plus Rough-Stalked Meadow Grass.

Timothy was not successful in the pasture owing to severe grazing by sheep and the effect of summer drought. It was much more successful and permanent in the hay sub-plots.

Cocksfoot is a splendid drought resister and invaluable where summer drought is usual.

Perennial Rye Grass, Wild White Clover, and Rough-Stalked Meadow Grass tend to hold Cocksfoot in check and prevent any coarseness in its growth.

The marked fluctuations in growth, as measured by the percentage ground covered, by the leading species in the plots is an annual occurrence of considerable regularity, the inter-specific competition being well shown by the graphs.

The close resemblance in results between the pastures and experimental pasture plots previously considered is a point of considerable importance.

The question of omitting Red Clover from seeds mixtures for long leys or permanent pastures, particularly where no hay cut is taken, is discussed.

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ON THE STRUCTURE OF THE IMMATURE STAGES OF THE FRIT FLY (*OSCINELLA FRIT* LINN.)

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(With 11 Text-figures.)

CONTENTS.

I. Introductory remarks.

The following paper deals with experiments and descriptions concerning the biology and structure of the immature stages of *Oscinella frit*, the frit fly of oats. The work has been carried out at the Rothamsted Experimental Station under the direction of Dr A. D. Imms.

A complete list of literature relating to *Oscinella frit* is not given because a summary of our knowledge of this insect, up to 1918, is provided by Collin (2). He includes (in his bibliography) thirty-three references to papers in addition to several full references given in the text. Since 1918 extensive investigations into the habits, prevalence and damage caused by *Oscinella frit*, and the possible use of resistant varieties of oats, have been carried out in England by Cunliffe, Fryer and others. In America Aldrich (1), in 1920, contributed a paper on the biology and habits of the European frit fly on wheat in that country. Frit fly attack in the stems of cereals is usually recognisable by the nature of the damage which is caused, viz. the central shoot usually dies and turns

brown whilst the surrounding shoots remain green. This is, however, not invariably the case, and where the characteristic signs of attack are missing it is necessary, for diagnostic purposes, to examine the morphology of the larva. Up to the present time no adequate description of any of the larval stages has appeared, and it is in an attempt to supply this deficiency that the present work has been carried out.

Technique.

The experiments were conducted in an outdoor insectary, which was fitted with glass at the front and top, whilst wire netting at each end allowed free circulation of air through the house; hence the experiments, whilst protected from rain, obtained as much sunlight as possible, and also a good supply of air.

For the purpose of rearing larvae, to work out the different stages in the life history, pots 11 inches in diameter containing insect-free soil were used. Oat seeds, variety "Supreme," were sown in two rows of six seeds each; the seeds in each row were approximately an inch apart, and the rows 2 inches apart, in order that the eggs might be detected without difficulty. The pots were protected with cages whose sides were covered with cellophane and the tops with muslin. Cellophane was found to be preferable to glass, since, while allowing the maximum entry of light, there was no condensation of moisture on the surface inside. The muslin-covered top gave access to the air.

In order to infect the experimental plants, mature insects were collected in tubes, in the numbers required, and introduced into the cages. In all experiments a small dish, containing sugar solution, was placed in each cage to provide nourishment for the insects.

To obtain first stage larvae, eggs were taken from the experimentally infected plants and placed on a piece of leaf blade, upon moistened filter paper, in a glass square. On hatching the young larvae bored into the leaf blade and were obtained by dissecting them out, under a binocular microscope. In order to investigate the tracheal system of the first stage larvae, very young individuals were taken which had not yet started to feed. These were obtained by placing eggs, in which the movements of the larvae were visible, into a moistened glass square. If moist filter paper is used the larvae, on emergence, burrow into it, and, being exceedingly small and almost transparent, are very difficult to detect. The tracheal system of all three larval stages was mapped out by placing living larvae, along with a small quantity of water, under a coverslip and gently pressing the latter.

Notes on the biology of Oscinella frit.

From the experiments that were set up to provide material for the study of the structure of the developmental stages, a number of general observations were made. These were supplemented by data obtained on the Rothamsted farm plots. In the experimental cages the eggs, in the majority of cases, were deposited inside the sheath at the base of the stem. The female insect protrudes the terminal segments of the abdomen and inserts the eggs within the fairly closely adhering sheath. In a few instances eggs were laid on the outside of the stem close to the surface of the earth; in the first leaf sheath or, rarely, on the base of a leaf blade. The number of eggs laid inside each sheath varied from one or two up to—in a few cases—thirteen, fourteen or fifteen, usually in groups. The majority of eggs kept under observation hatched in 3–4 days, but during colder periods some of them took 6–7 days. In the few cases where the eggs were laid on the bases of leaf blades, the larvae upon hatching mined through the mesophyll of the leaf in order to reach the shoot. Experiments carried out with plants at different stages of growth indicate that the heaviest infestation occurs in the two- and three-leaf stages. Eggs, however, were laid in moderate numbers on plants in the four- and five-leaf stages, but older plants were, with a few exceptions, neglected. Occasionally older plants, although not showing the characteristic external indication of attack, were found on dissection to contain larvae.

Fig. 1. Egg. × 155. *m.* micropylar area; *r.* longitudinal ridges.

II. The egg (Fig. 1).

The eggs are fusiform in shape, slightly curved and taper to each end. The posterior extremity is broadly round whilst the tapering is much more marked at the anterior end. The eggs vary in length from 0·58 mm. to 0·73 mm. with an average of 0·68; the maximum width ranges from 0·13 mm. to 0·20 mm. with an average of 0·16 mm. The colour, as seen with the naked eye, is glistening white, but appears creamy white when

examined in reflected light under the microscope. The surface of the egg is sculptured into a number of ridges and grooves which run roughly in a longitudinal direction; many of the grooves extend the whole length of the egg, whilst some bifurcate and a few end abruptly. At the posterior pole the surface is ornamented by a number of small polygonal areas. When examined under the high power of the microscope the ridges appear as long rows of closely apposed, bead-like papillae of thickened chorion, whilst the grooves are occupied by the softer portions of the chorion and have large numbers of papillae distributed over them.

In a median position, at the tapered anterior pole, there is a slight constriction which gives the region in front of it a cup-like appearance, when seen in surface view. The inside edges of the cup are directed backwards and inwards towards the constricted region, the centre of which is occupied by a depressed area of thinner chorion. The almost flat, posterior pole is strengthened by a circular area of thickened chorion.

Emergence of the larva.

The larva emerges through an irregular longitudinal slit at the anterior pole, slightly to the outside of the cup-like area. Wriggling movements of its body, and the scraping of the mouth hooks on the chorion, are continued for several hours before the larva finally emerges from the egg. This object is attained by the scraping action of the mouth hooks, facilitated by pressure produced by the movements of the enclosed larva, causing a fairly large slit to be made. The chorion, after the emergence of the larva, assumes a flattened, shrunken appearance and the exit opening is noticed as a roughly V-shaped slit.

III. The larval instars.

Three definite larval instars were observed, each showing, on careful examination, marked differences from the other. The main differences, apart from that of size which is not a sure indication (especially about the time of each ecdysis), are seen in the cephalo-pharyngeal skeleton and the spiracles. These are figured and described in a later part of this paper. The first stage larva has been taken in the act of emergence from the egg. Specimens have been obtained showing, respectively, the second stage larva still enclosed in the cuticle of the first stage; the third stage larva enclosed within the cuticle of the second stage; and also the cuticle of the third stage larva hardening to form the puparium. It will be evident, therefore, that errors in the determination of the respective instars have been avoided.

(*a*) *The first instar larva.*

The first instar larva varies in length from 0·7 mm. to 1·5 mm. with an average of 1·05 mm.; the maximum width ranges from 0·13 mm. to 0·23 mm. with an average of 0·16 mm. It is cylindrical in shape, rounded at the posterior end and tapering slightly towards the anterior end. It is almost transparent with the cuticle smooth, except at the junctions of the segments.

The head, which is somewhat retracted, is divided by a median depression into two symmetrical parts. It bears anteriorly a pair of two-jointed antennae; the basal joint is short and into its apex fits the globe-shaped second joint. Slightly posterior to these, and on the ventral surface, occur the maxillary palpi which arise as prominences from the integument. The maxillary palp consists of a group of rounded papillae of different sizes, each of which, in surface view, appears as a small ring with a dark central region; the group of papillae is bounded on its inner, posterior, and part of the outer margin, by a dark chitinous ridge. Between the anterior lateral margin of each maxillary palp, and the median depression, there is situated a single sensory papilla. Posterior to each maxillary palp, and on each side of the median depression, there is a ventral sensory organ. This structure consists of a chitinous ring in the centre of which are two adjacent rounded papillae, with a smaller one below them; the papillae are similar to those of the maxillary palp. The maxillary palp and ventral sensory organ, of each side, is enclosed within a long, oval-shaped area. A toothed chitinous plate, similar to that figured by Keilin (4) and de Meijere (5) for *Calliphora erythrocephala*, occurs on the integument at the right and left anterior margins of the mouth opening. According to Keilin this paired structure acts as a grater, and assists the buccal armature in lacerating the tissues of the host; in the first stage larvae of *Pegomyia winthemi*, where the buccal armature is feebly developed, these are strong hooks united to form a single grater (*vide* Keilin (4)). At the posterior border of the mouth, and close to the median line, is a pair of papillae.

The cephalo-pharyngeal skeleton (Fig. 2) is composed of paired sclerites; the most anteriorly situated of these are the mouth hooks whose apices project from the mouth opening. The mouth hooks are brownish yellow in colour and each has three sharply pointed teeth on its inner edge; the apical tooth is considerably larger than the other two which are similar in size and shape. Posteriorly to each mouth hook, and attached to it, is a long narrow accessory sclerite (*a.s.*); to its lower ventral surface is

articulated a second accessory sclerite in the form of a rounded nodule. Posterior to this latter sclerite, and articulated with it, is the intermediate or hypostomal sclerite which is completely fused with the basal or pharyngeal sclerite. The intermediate sclerite, though dark at its anterior end, becomes gradually paler and less sclerotised towards the distal extremity. It appears as an anterior process which broadens out

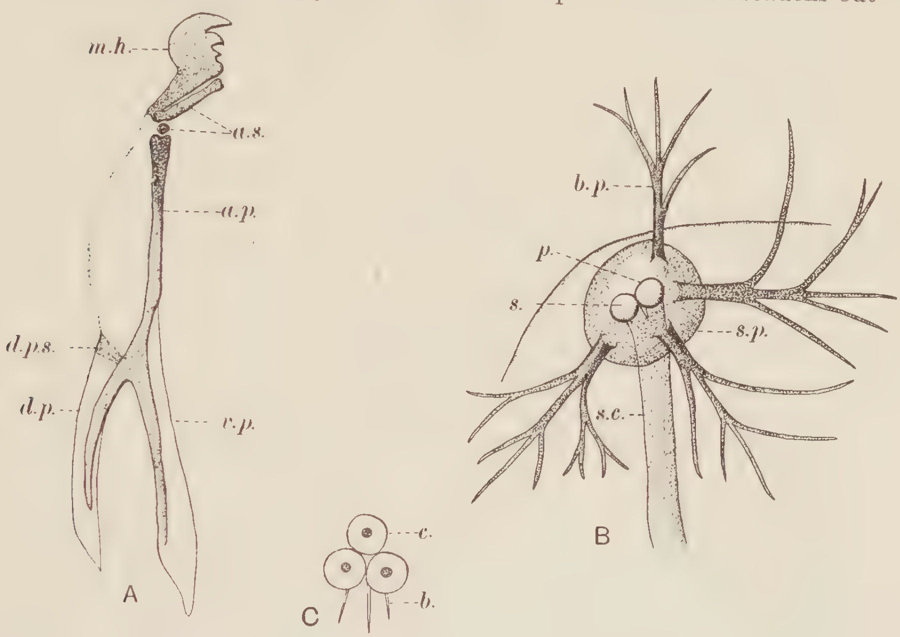

Fig. 2. A. Cephalo-pharyngeal skeleton of first larval instar ×375. *a.p.* anterior process of pharyngeal sclerite; *a.s.* accessory sclerites; *d.p.* dorsal process of pharyngeal sclerite; *d.p.s.* remnant of dorsal pharyngeal sclerite; *m.h.* mouth hook; *v.p.* ventral process of pharyngeal sclerite.

B. Posterior spiracle of first larval instar (surface view). ×1120. *b.p.* branched process; *p.* peritreme; *s.* spiracular opening; *s.c.* stigmatic chamber; *s.p.* stigmatic plate.

C. Organ on ventral surface of thoracic segments. Highly magnified. *b.* minute bristle; *c.* circle of cuticle.

posteriorly and passes into the pharyngeal sclerite; this sclerite bifurcates to form the dorsal and ventral processes, the latter being the longer. The anterior process (intermediate sclerite) isolates itself in the second stage larva as is the case with several other Dipterous larvae. The area (*d.p.s.*) which lies dorsal to the pharyngeal sclerite, near the region of bifurcation, is probably a vestige of the dorsal pharyngeal sclerite which, in some species, unites the anterior ends of the pharyngeal sclerites.

Body segments. There are eleven body segments, three thoracic and eight abdominal. The lines of junction of the segments are marked by circular swellings which are most prominent on the ventral surface; these, with the exception of the first two, are surrounded by transverse rows of minute cuticular denticles which are least numerous on the third segment. The rows, which are not continuous but occur in sets, are numerous on the ventral surface but gradually diminish in numbers towards the dorsal surface. The margin of the head and first thoracic segment is also surrounded by numerous rows of minute denticles. The thoracic denticles are long and pointed posteriorly, whilst those which occur on the abdominal segments have rounded apices.

About the middle of the ventral surface of each thoracic segment, and on each side of the median line, is an organ composed of three closely apposed circles (Fig. 2 C). The circles are arranged with one to the right, one to the left and the third behind, and each bears a minute seta. Keilin (4) has found similar organs, only differing in detail, in all Dipterous larvae which he has examined and, as they are in direct relation with the imaginal discs of the legs, maintains that they are vestiges of the ancestral larval thoracic feet. This interpretation has also been adopted by Pérez (6) in apodous Coleopterous larvae.

In a median position on the ventral surface of the eighth abdominal segment is a longitudinal slit, the anus; it is bordered on either side by a semicircular raised area. The whole is surrounded by a ring of chitin.

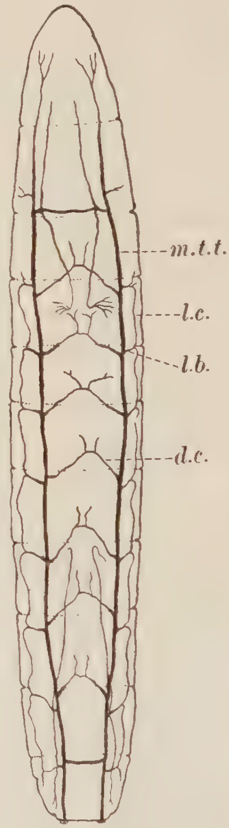

Fig. 3. Tracheal system of first larval instar. × 107. *d.c.* dorsal commissure; *l.b.* lateral branch; *l.c.* lateral commissure; *m.t.t.* main tracheal trunk.

Tracheal system. The first stage larva, like that of most *Cyclorrhapha*, is metapneustic and there is no indication of anterior spiracles (Fig. 3). The posterior spiracles occur at the apices of rounded prominences which arise as prolongations of the last abdominal segment. The openings of the spiracles are two in number, circular in shape, and each is surrounded by a ring of stiff chitin forming the peritreme. The spiracular openings lead through separate channels into the stigmatic chamber which, in turn, is in communication with the main tracheal trunk. The stigmatic

chamber may be distinguished from the adjoining trachea by the granular texture of the walls and the absence of spiral thickening. Situated laterally are four chitinised, hair-like structures each with a few branches. Seen in side view they have the appearance of supports to the membrane surrounding the spiracles.

In the first stage larva the main tracheal trunks run dorsally from the posterior spiracles along each side of the body, to terminate in fine branches in the first thoracic segment. The two trunks are connected by a dorsal commissure in each segment excepting the first. The first and last dorsal commissures are, however, much stouter than the others and

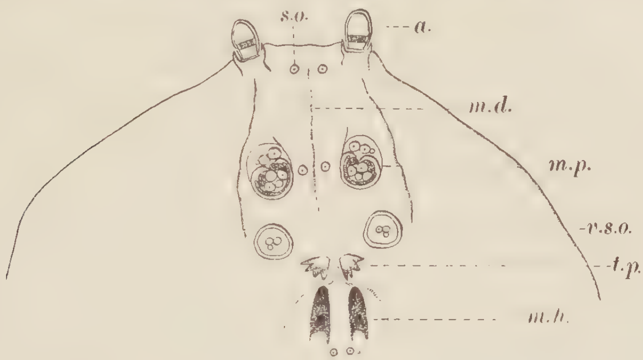

Fig. 4. Ventral view of head of second larval instar. ×560. *a.* antenna; *m.d.* median depression; *m.h.* apices of mouth hooks; *m.p.* maxillary palp; *s.o.* sensory organ between antennae; *t.p.* toothed plate; *v.s.o.* ventral sense organ.

run straight across from one longitudinal trunk to the other. The remaining commissures are slender and each makes a loop into the preceding segment; near the middle of each loop two small branches are given off anteriorly. Lateral branches are given off also from the main trunk, of which all but the first three are connected by a series of longitudinal commissures. From the latter, branches run out to the body wall.

(*b*) *The second instar larva.*

The second instar larvae vary in length from 1·7 mm. to 2·5 mm. with an average of 2·04 mm.; the maximum width ranges from 0·2 mm. to 0·4 mm. with an average of 0·28 mm. It is similar in shape to the first stage larva and, seen with the naked eye, is scarcely distinguishable from it. The head (Fig. 4) bears a pair of antennae, maxillary palps and ventral sensory organs, all of which are similar in structure to those of the

preceding stage. Situated between the antennae, and slightly posterior to them, is a pair of sensory papillae, one on each side of the median line. Toothed chitinous plates also occur at the anterior-lateral margins of the mouth opening.

The cephalo-pharyngeal skeleton (Fig. 5 A). The mouth hook has a large apical tooth; between this and the two posterior teeth is a small one which is not found in the first stage larva. The elongate accessory sclerite has undergone degeneration and become fused with the mouth hook; it is seen as a slight projection from the posterior ventral edge of the mouth hook. Immediately below the median projection from the ventral surface of the mouth hook is a small arc-shaped piece; this is probably the dentate sclerite to which is attached the mandibular depressor muscle. In this stage the anterior arm of the pharyngeal sclerite of the first stage larva has become differentiated in the intermediate or hypostomal sclerite. Between the anterior inner edges of the two intermediate sclerites (seen in ventral view) are two narrow sclerites which converge forwards and support the pharynx; close behind them is a cross-piece which appears to connect the intermediate sclerites. The pharyngeal sclerites have, at their anterior ends, two slender lateral processes which lie one on each side of the intermediate sclerites. The vestigial dorsal pharnygeal sclerite (*d.p.s.*) is present in the same position in the first stage larva. All the sclerites of the second stage larva are stouter, darker and more heavily chitinised than those of the first stage. The presence of the additional small tooth, the more rounded apices of the teeth, the **H**-piece between the intermediate sclerites and the isolation of the latter from the pharyngeal sclerites, are features which distinguish the cephalo-pharyngeal skeleton of the second stage larva from that of the first.

Body segments. Surrounding the margin of the head and first thoracic segment are numerous rows of minute hooks which, as in the case of the first stage larva, appear as irregular, broken rows. The rows of hooks which occur on the ventral surface at the junctions of the body segments are fewer in number than those of the preceding stage.

Tracheal system. The second stage larva is amphipneustic: the anterior spiracles are quite distinct and open to the exterior just in front of the posterior margin of the first thoracic segment. The stigmatic chamber expands at its anterior end and divides into five digitate processes (Fig. 5 B); at the distal end of each process is a circular area surrounded by a ring of chitin, the peritreme.

The posterior spiracles (Fig. 5 C) differ from those of the first stage larva in having three oval-shaped openings; these are connected through

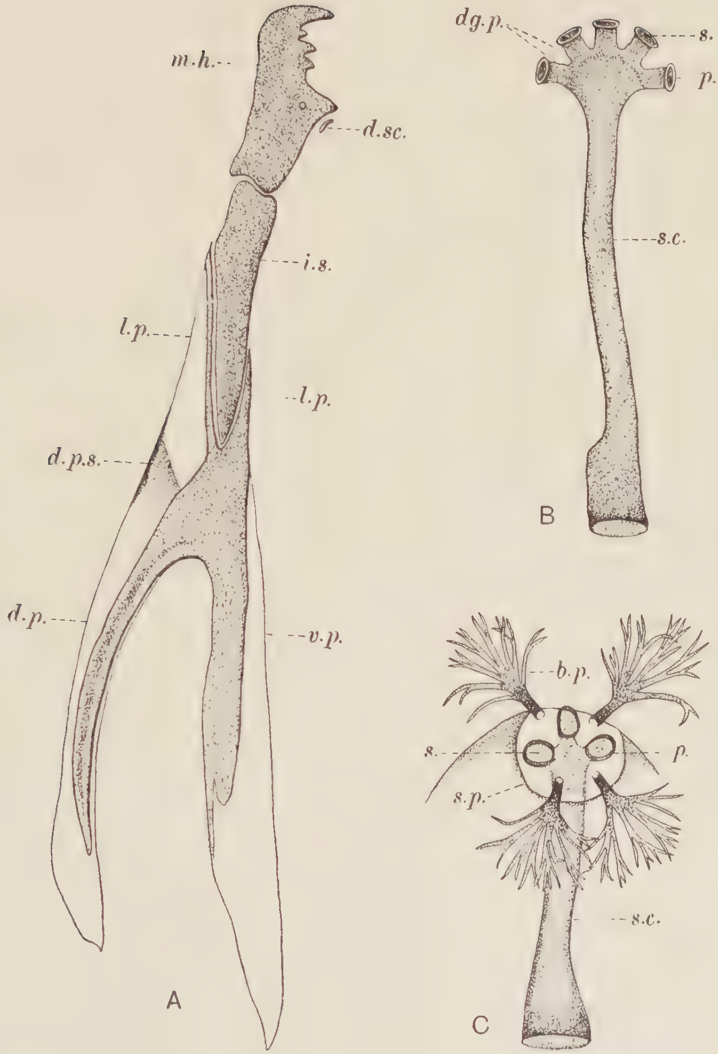

Fig. 5. A. Cephalo-pharyngeal skeleton of second larval instar. ×375. *d.sc.* dentate sclerite; *i.s.* intermediate sclerite; *l.p.* lateral process; other lettering as in Fig. 2, A.

B. Anterior spiracle of second larval instar (vertical view). ×1120. *dg.p.* digitate processes; *p.* peritreme; *s.* spiracular opening; *s.c.* stigmatic chamber.

C. Posterior spiracle of same (surface view). ×560. Lettering as Fig. 2 B.

a long stigmatic chamber with the main longitudinal trunk. The four branched processes are more ramified and alternate with the spiracular openings.

The internal tracheal system of the second stage larva (Fig. 6) differs little from that of the preceding stage; the main tracheal trunks, however, extend from the anterior to the posterior spiracles. The branching is also more profuse and several paired branches run forward alongside the pharynx in the first and second segments.

(*c*) *The third instar larva* (Fig. 7).

The third instar larvae vary in length from 2·8 mm. to 3·3 mm. with an average of 3·0 mm.; the maximum width ranges from 0·4 to 0·5 mm. with an average of 0·45 mm. The cuticle is firm whilst the larva has a yellowish colour due to the accumulation of reserve materials and is consequently more opaque than the first and second stage larvae. Apart from increase in size the head structures are very similar to those of the preceding larvae. The cephalo-pharyngeal skeleton (Fig. 8 A), though relatively smaller, is more heavily chitinised and shows further modification in detail. The mouth hooks are black; all the teeth have more rounded apices, and the second tooth from the anterior end is as large as the third and fourth. The intermediate and pharyngeal sclerites are heavily sclerotised; but, as in the preceding stages, they become much paler in colour towards the distal end.

Body segments. There are numerous rows of fine denticles around the margin, which separates the head and the first thoracic segment, but only a few rows of these structures occur along the junctions of the thoracic and abdominal segments.

Tracheal system. The anterior spiracles (Fig. 8 B) of the third stage larva normally terminate in six digitate processes although, in some cases, there are only five, as in the second stage larva; each process has a rounded apex, of similar structure to that of the preceding stage. The processes are, however, much longer and relatively narrower than those of the second stage larva. The posterior spiracles (Fig. 8 C) have three oval-shaped openings, each of which is connected by a separate lobe with the stigmatic chamber; the latter is continued in the main tracheal trunk (Fig. 9 B). In their general structure the posterior spiracles differ very little from those of the preceding instar. The internal tracheal system is also almost identical with that of the second stage larva.

Remarks on the cephalo-pharyngeal skeleton (Fig. 9 A). A transverse section, through the basal sclerite of the cephalo-pharyngeal skeleton,

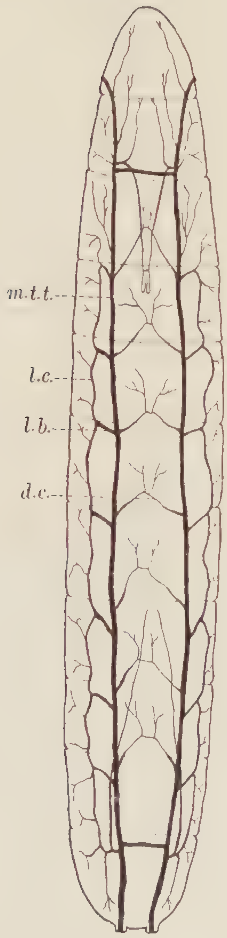

Fig. 6. Tracheal system of second larval instar. ×60. Lettering as in Fig. 3.

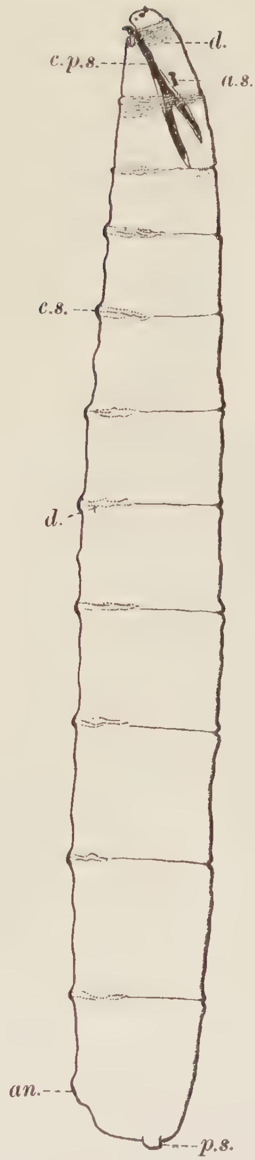

Fig. 7. Lateral view of larva in third instar. ×37. *an.* anus; *a.s.* anterior spiracle; *c.p.s.* cephalo-pharyngeal skeleton; *c.s.* circular swellings at the junctions of the segments; *d.* denticles; *p.s.* posterior spiracle.

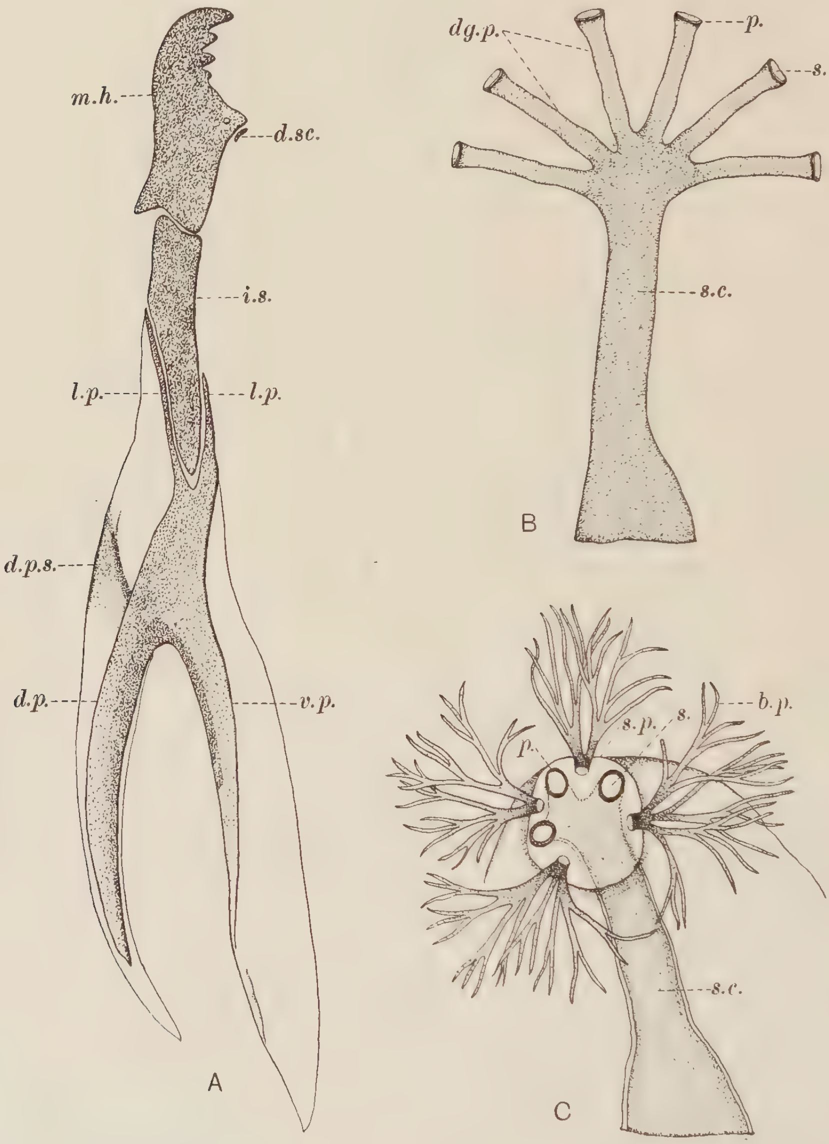

Fig. 8. A. Cephalo-pharyngeal skeleton of third larval instar. ×280. Lettering as in Fig. 5 A.

B. Anterior spiracle of third larval instar (vertical view). ×750. Lettering as in Fig. 5 B.

C. Posterior spiracle of same (surface view). ×375. Lettering as in Fig. 2 B.

shows a roughly oval-shaped opening, or trough, with a double ventral wall; the space between the two walls is the cavity of the pharynx. The whole trough is surrounded by a continuous cuticular rim, which is densest on the inner side, and this is also the case with regard to the cavity of the pharynx. The cavity of the trough, above the pharynx, has a lining of hypodermis which extends to the outer, upper ends of the dilator muscles. The capacity of the cavity of the pharynx is regulated by the dilator muscles which extend, from the upper wall of the trough, to the dorsal wall of the pharynx. An examination of a series of sections

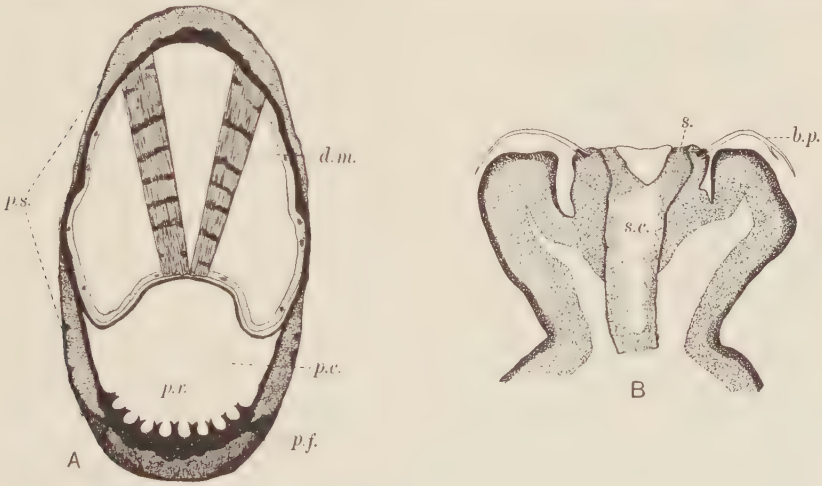

Fig. 9. A. Transverse section through pharyngeal sclerite of third larval instar. ×680. *d.m.* dilator muscles; *p.c.* cavity of pharynx; *p.f.* floor of pharynx; *p.r.* ridges in pharynx; *p.s.* pharyngeal sclerite.

B. Longitudinal section through posterior spiracle of third larval instar. ×600. Lettering as in Fig. 2 B.

(through the pharynx), from the anterior to the posterior end, shows a steady change in the structure of the ventral wall of the pharynx. At the anterior end the inner chitinised wall is smooth, but, as one proceeds backwards, a row of longitudinal projections, or ridges, protrude upwards into the cavity of the pharynx. These ridges, in the first few sections, are peg-like with a slight depression at their apices, but, in later sections, the depression is deeper and the ridges appear as Y-shaped structures. The lateral branches of these ridges (in some sections) almost meet, and break up the ventral surface of the pharynx into a number of small canals communicating with the rest of the pharynx by a series of slits. A few sections further back, the ridges disappear and the cavity of the

pharynx gradually decreases in diameter, especially in the dorso-ventral direction.

Keilin (4) has shown that the nature of the feeding habits of Dipterous larvae can, to a great extent, be determined by the presence or absence of these ridges in the floor of the pharynx. He maintains that all the parasitic larvae of animals, the carnivores, the predators, those which pass their whole life in the uterus of their mother (*Glossina* and *Pupipara*), and almost all the phytophagous larvae (gall-formers or miners) can be united into a vast ethnological group (larves biontophages). These larvae are deprived of ridges in their pharynx, and may be opposed to the saprophagous larvae, which have well-developed ridges. There are, however, certain phytophagous larvae, amongst which those of *Oscinella frit* have to be included, in which the ridges exist but in a form intermediate between the saprophagous and carnivorous types; the ridges are reduced and the ventral, inner wall of the pharynx is thick and heavily sclerotised. These transitional forms, Keilin is led to believe, are evolving actually under our eyes, are changing, or have changed their habitat, without their morphology having had the time to accomplish a complete cycle of transformation.

The phytophagous characters are more marked in the sclerites of the cephalo-pharyngeal skeleton described earlier in this paper. They are heavily sclerotised, the basal sclerite is deeply forked, and the dorsal and ventral processes are moderately long. The mouth hooks are toothed, whilst the surface of the head around the mouth is provided with paired dentate plates which augment the tearing capacity of the mouth hooks. A transverse section, taken near the apex of the latter, shows these dentate projections ("crochets supra-buccaux" of Keilin) at each lateral border of the mouth opening. Seen in section they resemble very closely the Y-shaped ridges which project from the ventral wall of the pharynx.

A comparison of the cephalo-pharyngeal skeleton, in the three stages, shows that this structure in the second stage bears a much closer resemblance to that of the third stage than to that of the first stage. The chief differences between the second and third stages is mainly a question of size; slight differences in form occur, but these are only in detail. There are, however, considerable differences between the cephalo-pharyngeal of the second and third stages and that of the first. The elongate accessory sclerite, which is attached to the mouth hooks in the first stage larva, seems, in the second and third stages, to have decreased in size and become fused on to the mouth hook. The dentate sclerite (Hewitt (3), Fig. 56, p. 134) which, in the first stage larva, lies between the base of

the accessory sclerite and the anterior process of the pharyngeal sclerite, appears in the second stage to have migrated forwards along the ventral edge of the mouth hook and occupies a similar position to that in the larva of *Calliphora erythrocephala* (Meijere (5); see his Figs. 157 and 158).

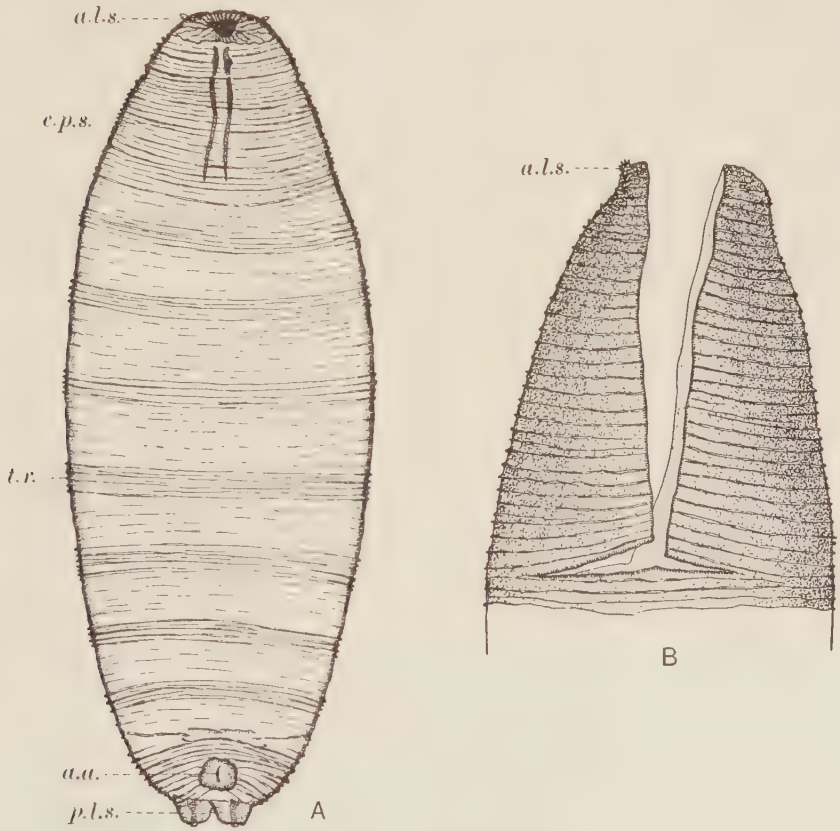

Fig. 10. A. Puparium (ventral view). ×37. *a.a.* anal aperture; *a.l.s.* anterior spiracles; *c.p.s.* cephalo-pharyngeal skeleton; *p.l.s.* posterior spiracles. *t.r.* transverse ridges.
B. Puparium, showing emergence slit. ×94.

A third important difference is seen in that the anterior process of the pharyngeal sclerite, of the first stage larva, has become separated in the second and third stages to form the H-piece or intermediate sclerite. A similar condition has been noted previously by Keilin (4) in the larval instars of *Pollenia rudis* (Keilin, Figs. 36, 37 and 38) and *Onesia sepulchralis* (Keilin, Figs. 49 and 51).

IV. The puparium.

The puparium (Fig. 10) is formed from the hardened integument of the third stage larva; it is reddish brown in colour, and when treated with a clearing agent the general outline of the pupal insect may be seen through it.

The puparium varies in length from 2·63 mm. to 3·08 mm. with an average of 2·8 mm.; the maximum width ranges from 0·73 mm. to 0·86 mm. with an average 0·8 mm. It is roughly barrel-shaped, tapering almost to a point at the anterior end, and is slightly flattened in a dorso-ventral direction. The integument is strengthened with transverse ridges of thickened cuticle; these are produced by the wrinkling and hardening of the integument of the third stage larva. (At the anterior and posterior poles the ridges of chitin are much thicker and harder.)

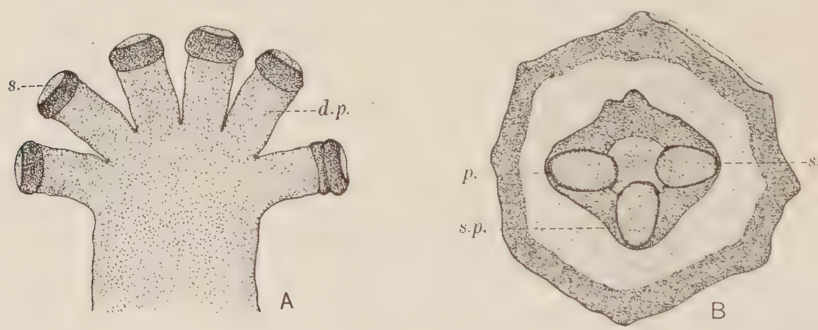

Fig. 11. A. Anterior spiracle of puparium (vertical view). × 900. Lettering as in Fig. 5 B.
B. Posterior spiracle of same (surface view). × 900. Lettering as in Fig. 2 B.

The anterior spiracles (Fig. 11 A) are situated at the apex of the puparium and slightly dorsal to the median lateral line. The six digitate processes are short and darker than those of the larva, especially around the distal ends which are deep brown in colour. The basal portion of the spiracle is brownish yellow.

The posterior spiracles (Fig. 11 B) are much like those of the third stage larva. The three oval openings are situated on a dark yellowish brown spiracular plate, which is wide between the slits, but has a narrow margin at the outer edge of each. Surrounding each spiracular plate, but with an area of paler chitin in between, is a firm ring of dark brown cuticle.

Emergence of imago from puparium. The adult, unlike the majority of Cyclorrhaphous flies, emerges from the puparium through a horizontal,

narrow V-shaped slit (Fig. 10 B) which extends round the anterior end and backwards along each side. The pressure exerted by the imago, in emerging, causes fractures to extend from the base of the slit around the circumference of the puparium. The extent of these slits is very variable and, if pressed under a coverglass, the two portions, viz. the dorsal area bearing the two laterally situated anterior larval spiracles, and the ventral area bearing the cephalo-pharyngeal skeleton, become detached from the puparium.

V. Summary.

1. The morphology of the immature stages of *Oscinella frit* are described and figured and certain observations of a biological nature are recorded.

2. The egg is described together with the method of eclosion of the first instar larva.

3. The structure of the larva in its three instars is described in detail with particular reference to the cephalo-pharyngeal skeleton and the spiracles.

4. The first instar larva differs markedly in structure from that in the two instars which follow. Larvae in the second and third instars are distinguishable, apart from size, only in small structural details.

5. The puparium is described, with particular reference to the spiracles, together with its method of dehiscence during the exit of the imago.

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ON THE ECOLOGY AND CONTROL OF SLUGS

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PART I. ECOLOGY.

I. INTRODUCTION.

SLUGS are perhaps the most persistently injurious of the many pests which occasion direct loss to both farm and garden crops. When crops are present and weather conditions are not severe slugs feed throughout the year, taking an almost inestimable toll of practically all agricultural produce. Their insidious habits tend to obscure their importance, and it is usual that their depredations pass almost unnoticed until they assume epidemic proportions.

During recent years the slug pest has been increasingly important in the north-west of England, particularly in south and east Lancashire and north Cheshire. The present account concerns investigations carried out in the Department of Zoology (Agricultural Entomology) of the Victoria University of Manchester. The cost of the investigations has been met by a grant from the Ministry of Agriculture. Acknowledgments are due to Prof. J. S. Dunkerly for his constant interest in the work and willingness to discuss lines of investigation, to Mr J. C. F. Fryer, M.A., for his helpful criticism of the MS., and to Mrs M. Miles, M.Sc., for assistance with field work and considerable help in working through the mass of data collected during the period of the investigation.

II. Local conditions.

The region from which observations have been drawn extends from Altrincham and Manchester in the south to Preston and Burnley in the north, special attention being given to the east Lancashire area referred to geologically as the Rossendale anticline. North Cheshire, comprising the Altrincham, Northenden and south Manchester areas, and extending along the south of the Mersey to Runcorn, is devoted largely to horticulture and market gardening. On the north of the Mersey the belt of intensive agriculture extends almost to Liverpool, and then swings northward to Ormskirk where agriculture is highly specialised. After a slight break for general farming to the north of Ormskirk, intensive culture and market gardening are again important in the Preston district and parts of the Ribble Valley, this area merging into the Fylde and Blackpool region on the west and the Rossendale district on the south-east.

The Rossendale district lies between this western belt of agricultural land and the high hills of the Pennines. The country is largely moorland, and the agriculture consists chiefly of stock rearing and grazing. For over fifty years there has been little tillage, but to meet the exigencies of the Great War, considerable areas in the vicinity of the large industrial centres were broken up for allotment cultivation. It is in the allotment areas and market gardens about the large industrial towns that the slug problem is most acute. In east Lancashire it seems that during the first two years after the land was broken up there was comparatively little loss from slug attack. By the third and fourth years the slug population had increased to such an extent that definite losses were noted, and by the sixth year it became evident that cultivation must be restricted to such crops as seemed able to withstand the depredations of slugs.

1. *Soils.*

The soils of the region under consideration show considerable variation.

The Rossendale area consists of carboniferous rocks, with millstone grit and coal measures largely predominating. As a result of glaciation there are thick deposits of boulder clay, with here and there sand and gravel. Two distinct soil types are recognisable, both having a matrix of stiffish clay, with limestone and chert boulders plentiful in one case, but comparatively rare in the other. From a preliminary study of the soil of this area, Smith (15) describes it as a greyish black sticky loam,

changing abruptly at about 3 inches to a granular, multi-coloured, sandy clay. At 6 inches below the surface mixed greys and rust colours predominate, then the texture may become more open or more compact, since the drift material varies rapidly from a sandy clay to a sticky sand.

In the western and southern agricultural belts the soils are extremely varied. A good deal of glacial material was deposited in the lowlands; clays, sands and gravels are intermixed as a result of river activity, and considerable deposits of alluvia are common, together with extensive peat mosses. The soils are generally warm brown to reddish brown, according to the organic matter content and the nature of the subsoil. They range from sands to clays, with sandy loams and heavy loams much in evidence, and Smith (*op. cit.*) states, "It is not uncommon to make a series of eight or ten borings in the course of a traverse of a few miles without finding any are duplicated with regard to nature and sequence of strata." The soil of the mosslands is fairly constant in character, consisting usually of a surface layer of 6 to 8 inches of black, decomposed organic matter, overlying a brown peaty material of considerable depth. Where the peat is thin sand may enter into the composition of the soil.

The agriculture of the region follows the broad soil divisions, permanent grassland on the slopes of the Pennines, general farming in the lowland areas with the glacial drift soils, and intensive culture with market gardening on the peats, sandy peats and alluvial sands.

2. *Climatic conditions.*

The climatic conditions in the region are directly influenced by the proximity of the sea on the west and the Pennines on the east. The rainfall is generally heavy, ranging from about 28 inches on the western lowlands, to 40–60 inches in the east at an elevation of 800–1000 feet. The average annual rainfall for east Lancashire during the past eighty years is 47·3 inches, and the average relative humidity for the same period is 83 per cent. The temperature ranges from about 40° F. in winter to about 60° F. in summer, the average for the past eighty years being 47° F.

III. SLUGS IN LANCASHIRE.

1. *Species of economic importance.*

The slug species of economic importance in Lancashire belong to two families: *Agriolimax agrestis* L. and *Milax sowerbii* Fér, members of the family Limacidae Gray; and *Arion hortensis*, *A. ater* and *A. subfuscus*, belonging to the family Arionidae Gray.

Agriolimax agrestis L., the grey field slug, is probably the best known species, since it is widely distributed throughout the British Isles. Two varieties have been commonly observed in Lancashire, var. *reticulata* and var. *pallida*, the former occurring in greater numbers. *A. agrestis* is mottled grey in colour, often tinged with reddish or yellowish, and with a more or less readily discernible respiratory pore on the right side, towards the posterior region of the mantle. It is omnivorous, feeding upon almost any kind of organic matter, but shows a preference for the leafy parts of plants, though stems and roots may also serve for food. It is a prolific species, active and hardy, and will feed in mid-winter when the temperature is only a few degrees above freezing-point.

Milax sowerbii Fér. has the body acutely keeled along the mid-dorsal line, from behind the shield to the top of the tail. The body is usually dark brown or dark grey, and against this ground colour the keel is fairly readily discernible on account of its lighter colour. The mantle has a characteristic lenticular groove, and the respiratory pore is situated just behind the centre of the mantle on the right side. *M. sowerbii* is most abundant in the Manchester district, and is distinguishable from the closely allied species, *M. gagates*, by the prominence of the pale keel and by the fact that on dissection the long tapering spermatheca of *M. sowerbii* contrasts strongly with the more globose spermatheca of *M. gagates*.

Arion hortensis Fér. is much smaller than the other species commonly met with. It varies in colour from brown to black, and is distinctly marked on the back and sides with longitudinal darker bands. The foot is yellowish in colour and the slime is also yellow. Like that of *M. sowerbii* the skin of *A. hortensis* is exceedingly tough, as distinct from the softer and more fleshy skin of *A. agrestis*.

Arion subfuscus Drap. is smaller than the closely allied species, *Arion ater* L., the large black slug commonly found about hedges and damp ditches, and not usually recorded as an injurious species. *A. subfuscus* may be distinguished from *A. ater* by its shorter and flatter rugosities, and by the fact that it cannot assume the almost hemispherical shape which is a characteristic feature of *A. ater*. The variety of *A. subfuscus* most commonly observed in Lancashire is *fuliginea* Mor., sub-variety *brunnea*. It is a lightish coffee brown in general coloration, the dorsum and shield being rather darker, the lateral banding dark and the foot yellowish. The slime is also yellowish to brown. Young specimens are dusky yellow.

2. *Distribution of the various slug species.*

Agriolimax agrestis and *Arion hortensis* appear to be generally distributed in the region under consideration, and usually occur in approximately equal numbers. The character of the soil seems to have little influence on their distribution, the slugs being equally abundant on clay, sand, alluvium and peat. *Arion ater* is also generally distributed but is confined to rural areas, its occurrence about industrial centres being rare. *Arion subfuscus* is common on the clay soils about the industrial areas of east Lancashire, and seems to be most usually taken on grassland, newly broken up grassland, or in the neighbourhood of stacked turf. *Milax sowerbii* seems to be associated with the boulder clay and occurs abundantly in the Manchester district in the allotment areas on this type of soil. Even when the surface soil appears to be of a lighter character, the subsoil is usually heavy clay if *M. sowerbii* is present. This slug has usually been regarded as essentially a town-dwelling species, but its range appears to be extending, and it is interesting to note that where it occurs in association with other species, such as *A. agrestis* and *A. hortensis*, *M. sowerbii* is always the dominant species.

3. *Life history.*

Slugs are soft-bodied molluscs. The greater part of the body comprises the foot, an elongate, muscular structure with a flat under-surface, which functions as an organ of locomotion. At one extremity is a blunt head which bears two pairs of retractile tentacles and the mouth, the latter being placed beneath the tentacles. The mouth consists of a fleshy upper lip, below which is a chitinous, file-like organ, the radula, used to scrape the food material. Behind the head is a somewhat irregular oval mass, the mantle, which lies over the internal organs and is associated with the small rudimentary shell. The body tapers posteriorly.

On the right side of the mantle is an orifice which is connected with the respiratory chamber lying beneath. The reproductive organs lie on the right side, and the genital aperture is situated between the respiratory pore and the base of the right tentacle. In the skin are numerous mucus glands which secrete the slime which envelopes the body. Beneath the internal organs is a large slime gland, which opens just below the mouth and secretes the slime over which the slug moves (3).

Slugs are hermaphrodite. Although self-fertilisation has been observed by Wooton (19) it does not generally take place. It appears to be prevented by the ripening of ova being delayed until spermatozoa are no longer produced. Thus slugs are first functional males and later

females. In some species the genital organs undergo a series of successive metamorphoses, and only subsequently become bisexual, or remain unisexual owing to the atrophy of some of the sexual organs.

The life histories of the various species of slugs occurring as pests do not seem to have been fully observed in this country, but from available records the outlines appear similar. Taylor (16) has collected most of the available information, Theobald (17) has described the species of economic importance, and Miles (10) has given an account of *Agriolimax agrestis* and recorded further details of its life history (11).

(*a*) *Agriolimax agrestis* L.

Mating takes place on the surface of the soil when the weather is warm and moist. After mating the slugs enter upon a quiescent period and about three weeks later eggs are laid. The eggs are translucent, spherical in shape, and about $\frac{1}{12}$ inch in diameter. They are deposited in clusters of eight to sixty eggs, in damp situations, either in the soil, among decaying root fibres or in moist vegetation. Each egg consists of a true ovum embedded in a gelatinous substance which is surrounded by a membrane. As embryonic development takes place the opaque white mass in the egg increases in size until the general form of the slug is recognisable. A few days prior to hatching the young slug becomes active and eventually ruptures the membrane of the hyaline material enclosing it (11). After eclosion the young slug feeds upon any available organic matter. Conditions of temperature and food supply determine the length of time required to reach maturity, but this is not usually more than six to eight months. Mating and egg-laying take place at intervals throughout the year, but eggs laid in the late autumn may not hatch until the following spring. *A. agrestis* is very prolific and it has been estimated that each functional female lays about 1000 eggs. Two specimens kept under observation laid over fifty eggs before those first laid had hatched.

(*b*) *Milax sowerbii* Fér.

Milax sowerbii is a subterranean species which has been considered gregarious because of the common occurrence, in the autumn, of numbers of the slugs together under stones, in the soil, among root fibres and in decaying vegetation. This gregarious habit does not extend to their feeding and seems to be chiefly associated with hibernation. Mating is most readily observed in the late summer and autumn when the slugs congregate, but it seems evident that it is not confined to this time of the year. The eggs are transparent, somewhat ovoid, and comparatively

large, being 4–5 mm. long and 3 mm. wide. They are deposited beneath the surface of the soil in small clusters of from four to twelve or thirteen, and are held together by a transparent mucus. Incubation requires about four weeks.

Observations were made on the embryonic development of *M. sowerbii.* Five or six days after the eggs were laid a dark area became discernible in the centre of the egg. As development proceeded this area enlarged, and in about three weeks assumed a definite slug form, head, tentacles, eye spots and caudal region becoming distinguishable. Two or three days prior to hatching movements could be detected, and from ten to twenty hours preceding emergence they became more violent, and the radula was rasped continually against the enveloping membrane. Ultimately this membrane was ruptured, the head of the slug was protruded and the body gradually withdrawn.

On eclosion the young slugs measure approximately 4·5 mm. in length and 1 mm. in width, and are creamy white in colour. After two to three weeks the slugs become dull grey, the keel becomes acute and the margin of the pallial gutter is filled with pigment. As development takes place the slugs gradually assume adult coloration.

(*c*) *Arion subfuscus* Drap.

The eggs of this species are laid in the soil or among decaying vegetation, in clusters of from about twenty to over fifty, held together by a translucent, slimy mucus. They are almost spherical in shape, and measure from 2·5 to 2·8 mm. in diameter. When newly laid they are milky white and semi-pellucid, with the enveloping membrane slightly granular. After three or four days they become opaque white owing to the deposition of calcareous particles in the membrane. This calcareous deposit renders impossible any observations on the development of the embryo.

A batch of eggs laid on September 18th hatched between October 21st and 22nd. The heads of the young slugs were thrust through the membrane of the eggs and the whole body then drawn slowly out, leaving a cluster of shells which looked rigid but which broke down at the slightest touch. The newly emerged slugs were a semi-translucent white. They were long and slender with a finely tapering body, and measured from 2·75 to 3 mm. in length. The eye tentacles were short and the eyes were clearly visible about half-way along the tentacles. The shell was distinctly visible on emergence, and within two days the rugosities became well marked and the respiratory orifice could be seen. The first indication of

colouring occurred with the development of the dark lateral banding which is characteristic of the species, but the general body colour changed very gradually to the typical coffee brown of the adult. Compared with the rate of growth of *Agriolimax agrestis*, the growth of this species is slow. Twenty-eight days after emergence the average length of ten slugs was only 6·3 mm., and after three months it was 11·8 mm. Three months after eclosion there was considerable differences in the size of the slugs, one measuring 18 mm. and another only 5·5 mm. As development continued these differences became less apparent and the slugs gradually approximated one another in size.

Observations were made on the feeding habits of the young slugs. On the first and second days after eclosion unbroken leaves were left untouched, and the slugs fed on humus or the broken edges of leaves. When three days old the slugs began feeding on the unbroken lower epidermis of cabbage leaves, and large areas of the mesophyll were eaten out, leaving the upper epidermis intact. Mature slugs are prodigious feeders, and, according to Taylor (16), in the autumn display a marked preference for fungi.

The life histories of these three species of slugs bear fairly close resemblance to one another. No observations have been made on the life histories of *A. hortensis* and *A. ater*, but there is no reason to suppose that they depart to any great extent from the general lines of those of the species already dealt with.

4. *Feeding habits.*

Certain feeding habits appear to be broadly characteristic of particular slug species, and certain preferences for plant species and parts of plants are exhibited. *Agriolimax agrestis* is usually a leaf and stem feeder and shows a distinct preference for feeding above ground. *Milax sowerbii*, on the other hand, is of subterranean feeding habit, and confines its attacks largely to the underground parts of the plant. Roots and underground stems suffer most severely, and it is common to find in urban gardens and allotments, potato tubers so riddled with holes as to present the appearance of a sponge. *M. sowerbii* is considered by some writers to drag material into the soil to devour later, but this has not been observed by the writers. It has been noticed, however, that while *A. agrestis* normally feeds but little during the day, *M. sowerbii* continues to feed below the surface of the soil throughout the day, and has been observed feeding within a quarter of an inch of the surface of the soil on hot, sunny days in midsummer. *Arion hortensis* feeds fairly generally at

or below the surface of the ground and commonly tunnels into the bases of succulent stems, roots and tubers. It is frequently destructive to herbaceous plants, feeding well down near the base and gnawing at the young succulent growth as it develops. Paeonies and Solomon's Seal seem especially liable to attack and may be extensively injured.

Consideration of the distribution of the various species in conjunction with their feeding habits gives some indication of their economic importance. Being widely distributed, *A. agrestis* is injurious in both town and country gardens and also on farms, but it is mainly associated with stem and leaf injury, from which the plants may ultimately recover. Cereals may be badly attacked, but their vitality enables them to throw off the effects of attack once the intensity is decreased.

M. sowerbii, being predominantly urban in distribution, is most injurious in gardens and allotments, and readily attacks bulbs, seedlings and root crops. Loss due to attacks on seedlings may be serious, as a single slug may destroy numbers in a night. Pulse crops seem to suffer severely, peas and beans being sometimes so injured below the ground level that few shoots appear above ground. With runner and dwarf beans, the radicle and plumule may be destroyed as soon as they protrude, and when the cotyledons lose the protection of the testa, they too may be devoured. *Arion hortensis*, which is more generally distributed than *Milax sowerbii*, is associated with similar injury, particularly in rural localities.

Although these differences in feeding habits may be observed, under certain circumstances such as the presence of a high slug population, all species will feed indiscriminately on any part of the plant—root, stem, leaves and flowers. Plants which are earthed up are especially liable to attack by slugs because the loose soil permits the animals to move about freely. Celery may be so disfigured by attack as to be unmarketable. Brassicas, particularly when planted on land which has been fallow for a few weeks, may suffer serious injury. The injury may take the form of complete severence of the head just below the base of the leaf stalks, or, if the plants have been set rather deeply, the stem may be attacked below soil level and rasped away until the plant is only held by a few strands of central vascular tissue. Occasionally the terminal bud is destroyed by slugs and the plant becomes "blind" or develops a multiple head.

Farm crops are also liable to slug attack. Cereals, particularly on low-lying, badly drained or heavy soils, are most usually attacked and root crops may also suffer. Potatoes may be attacked in the autumn

both in the ground and in the clamps. Seedling swedes may be attacked, and occasionally swedes are injured in storage.

IV. Observations on the factors determining slug prevalence.

1. *Observations on the slug population of a limited area.*

The nocturnal and crepuscular habits of slugs make it very difficult to form any estimate of the slug population of a particular area, especially where the slugs migrate some little distance between the feeding sites and shelter. Some figures which may be taken to reflect conditions of slug population have, however, been collected in a garden in Manchester. The garden is approximately a quarter of an acre in extent. On three sides it abuts directly on city streets from which it is separated by a low hedge. On the fourth side are the walls of two dwelling houses and a boundary wall about 6 feet high separating the garden from other small urban gardens or back yards. The garden receives the usual cultural treatment for the district, is well kept and produces a variety of market-garden crops.

The owner of the garden has trapped slugs persistently since he took possession in 1925. In 1925 the garden received a dressing of lime at the rate of 6 tons per acre. During that year 47,185 slugs were collected under traps consisting of boards, sacking, stones and cabbage leaves. In 1926 the number of slugs collected was 35,952. In 1927 the number increased to 61,304 and in 1928 49,908 were obtained. In 1929 the number of slugs collected in the traps was 8377 and in 1930 the number collected during the year was approximately 10,000.

While the above figures give no indication of the slug population of the garden, they serve to indicate fluctuations in the numbers of slugs present. From a study of these fluctuations correlated with a study of the weather during the period, it would seem that fluctuation in the slug population is a direct response to general climatic conditions. In 1925 the range of temperature recorded at Whitworth Park, Manchester, about half a mile from the garden, was from 21° F. to 89° F. and the mean daily temperature for the year 49·1° F. These temperature conditions approximate to normal and the rainfall was 34·88 inches, slightly above normal. During June the weather was dry and hot, and there was a prolonged period of very cold weather during November and December. The drought and heat in the early summer would have little effect on mature slugs but might be expected to check mating and egg-laying and

increase the mortality among young slugs. Similarly the cold weather towards the end of the year would have practically no influence on the numbers of slugs caught in 1925 but might be expected to have some adverse effect on the slug population of the following year. In the garden under observation the numbers of slugs captured during 1926 showed a decrease of about 25 per cent. from the figures for 1925.

1926 was a year of mild, rather damp weather. The rainfall was 33·75 inches, only slightly above normal. The range of temperature, 24° F. to 85° F., was less than the preceding year but the mean daily temperature, 50·1° F., was higher. Such climatic conditions appear favourable for slug development, and in the next year when the results of the previous year's favourable conditions would be most apparent, the number of slugs caught at the traps showed an increase of about 70 per cent.

The year 1927 was wet and cool. The summer temperature did not rise above 78° F. and the mean daily temperature was below normal. Such conditions may be considered fairly favourable to the development of slugs, and this was reflected in the high numbers caught during that and the following year.

The year 1928 was wet, the rainfall being 38·10 inches, a matter of 6 inches above normal for the district. The temperature ranged between 22° F. and 79° F., and the mean daily temperature, 49·5° F., was approximately normal. The slug population may therefore be considered as living under favourable conditions, and the number of slugs collected from the traps was nearly 50,000. The effects of the 1928 weather conditions are not apparent in the number of slugs captured in 1929 owing to the influence of unusually severe weather at the beginning of that year.

In 1929 the total rainfall for the year was about normal and the mean daily temperature was only slightly below normal. It was, however, a year of great fluctuations, the range of temperature being from 15° F. to 85° F., and the rainfall being deficient during the first nine months and excessive during the last quarter. In January and February there were prolonged periods of intense cold, and these, combined with almost continuous drought, seemed to have an adverse influence on the slug population over a considerable area. In the garden under observation the numbers of slugs collected from the traps was 8377, only about one-sixth of the number caught the preceding year.

The year 1930 was on the whole cool and wet, though in the early spring the weather was fairly dry. Conditions for slug development were generally favourable, and in spite of the widespread reduction in the slug population, owing to the adverse conditions of 1929 and the

persistent trapping which has been carried out for a number of years, the number of slugs appears to be increasing rapidly.

From this study of the slug population in a limited area only tentative conclusions can be drawn at present. It would appear, however, that localised treatment aimed at the destruction of slugs has no measurable effect on the slug population of the area, even when carried out consistently over a period of years. It also appears that especially favourable conditions for slug development, such as a mild wet season, may lead to a considerable increase in the slug population in spite of such operations as continuous trapping. Adverse weather conditions seem to have a marked effect on the slug population, but it would appear that the slug population rapidly increases towards what might be termed "normal" once conditions are again usual, and that an operation like trapping is ineffective to check this increase.

2. *Natural factors influencing slug development.*

(*a*) *Moisture.*

Moisture is one of the most important factors influencing slug development. Large quantities are taken into the body in the food and utilised for the production of slime which is essential for locomotion (3) and enables the skin to perform its respiratory functions.

External moisture is also essential to the well-being of slugs. Night is the period of greatest slug activity, and at this time the relative humidity of the atmosphere near the surface of the soil is very high, often reaching saturation point. During the day when the relative humidity of the atmosphere is much lower, slugs shelter in the soil where the air is always saturated or nearly saturated with water vapour (6) or in damp vegetation where conditions of humidity are similar. Only in wet weather are slugs found crawling over the surface of the soil or feeding openly during the day. Dry atmospheric conditions react unfavourably on slugs. Moisture from the surface of the body is evaporated and this necessitates the continuous exudation of slime to maintain the body in its normal moist condition. If this loss of moisture is prolonged the slug becomes exhausted and may die.

The influence of moisture on slug development is apparent from a comparison of slug activity in different years. During dry seasons crop injury is reduced to a minimum and usually occurs only below the level of the ground, while during wet seasons injury is widespread and serious. Prolonged spring droughts have a marked effect on slug population, since moisture is essential for the hatching of the eggs and the survival of the

young slugs. During the cold dry spring of 1929, slug activity was so checked in the district under consideration that injury to the crops was scarcely apparent until late in the summer when moisture conditions were becoming normal.

(*b*) *Temperature.*

It is generally accepted that slugs in protected sites are able to withstand extremes of temperature, hence a few degrees of frost are not normally lethal, neither are protracted periods of high temperature when moisture and other environmental factors are favourable. Both *A. agrestis* and *M. sowerbii* have been found to feed throughout the winter except when the temperature was below freezing-point. On the approach of frost slugs seek shelter in the soil, or beneath refuse, pots, plants, etc. In one instance in February 1929, forty-one slugs were found under a tub of soil after hard frost had been continuous for several days. All the slugs were frozen quite stiffly. When taken into the laboratory and gradually thawed, the thirty-seven specimens of *M. sowerbii* were found to have been killed by the frost, but of the four specimens of *A. agrestis* only one had been killed. This suggests that *A. agrestis* is better able to resist low temperatures than *M. sowerbii*, and may account for the remarkable decrease in the number of slugs caught after the severe frosts of 1929 in the garden in Manchester (p. 380) where the slug population was almost exclusively *M. sowerbii*.

When the approach of low temperature is gradual there is a gradual response on the part of the slugs and the survival possibility is consequently greater, but with a sudden lowering of temperature the possibility of survival is greatly reduced owing to the slugs still sheltering in unprotected situations. A simple experiment was devised to demonstrate that slugs worked their way deep into the soil in direct response to the lowering of the temperature. A glass cylinder 6 inches in diameter and 2 feet long was filled with soil and ten specimens of *M. sowerbii* were placed on the top of the soil. By means of a freezing mixture applied to the top of the cylinder, the soil was gradually frozen to a depth of 3–4 inches. Within twelve hours seven slugs had penetrated well below the limits of freezing and survived the experiment. The three which failed to penetrate below the region of low temperature were frozen and died. The lethal point appeared to be between $-1°$ C. and $-2°$ C., and it was observed that when once slugs of the species *M. sowerbii* were frozen stiffly they failed to revive with a rise in temperature.

How far the severe weather of the winter of 1928–29 was responsible

for the great reduction in slug prevalence in south and east Lancashire in the spring and early summer of 1929, it is impossible to determine accurately, but it would appear that its influence was considerable. Other investigators have found that slugs were affected adversely by frosts: Hawley (7), writing of slug intensity after an abnormally severe winter (1917–18) in New York, states "Few slugs survived.... Very few of the millions of eggs deposited under the bean poles in the fall of 1917 survived."

(*c*) *Organic matter.*

The presence of organic matter has a dual importance in relation to slug development. Variation in the water content of similar soils follows closely the differences in the amount of organic matter present (14). Because of its water-holding capacity the presence of organic matter encourages slug development, and since it also serves as food for slugs at various points in the life cycle its influence is increased. Newly hatched slugs feed at first on undecomposed organic matter and broken, damaged plant tissue. In the early spring, before crops are available, the organic matter in the soil appears to be one of the main sources of food for young slugs, and during the winter when conditions are unfavourable for surface feeding and metabolic processes are reduced, the organic matter in the soil also provides food for the adults. The importance of organic matter in the soil was demonstrated in the laboratory when newly hatched slugs were kept alive for over six months under conditions in which moisture and organic matter only were present.

(*d*) *Soil reaction.*

From time to time it has been suggested that a relationship exists between the soil reaction and the number of organisms present in the soil, and the idea that harmful organisms are favoured by acid conditions is popular. In their work on snails, however, Atkins and Lebour (2) have concluded that snails definitely prefer alkaline conditions.

Field observations made in various parts of Lancashire under a wide range of soil conditions suggested that soil reaction had little direct bearing on the slug population in so far as it was indicated by the amount of slug damage present among the crops. Soils of similar pH value showed marked differences in the amount of slug injury to the crops, and areas where serious slug injury occurred showed considerable variation in pH value. On soils with a low degree of acidity, pH 6 to 6·5, losses owing to slug attack were extensive and usually serious. On allotment gardens on the boulder clays and drift soils in east and south-

east Lancashire the pH was about 4·5 and slugs were widely prevalent and crop injury serious and persistent. On the peaty soils of south Lancashire and north Cheshire similar conditions of high acidity prevailed, but the injury caused by slugs was so slight that the pest could not be considered of importance.

From these field observations it was apparent that in Lancashire and Cheshire lime status had comparatively little influence on the prevalence of slugs since the injury assumed similar proportions on acid soils, on soils approaching neutrality and on soils with an alkaline reaction. It seems possible, however, that this apparent indifference to conditions of acidity might be the result of the predominating influence of such favourable factors as suitable moisture conditions, the widespread use of organic manures in the areas studied, the general prevalence of heavy soils and the cultivation of susceptible crops. The low slug infestation which was noted on peaty areas seemed to be the effect of factors other than lime status.

3. *Influence of cultivation on slug prevalence.*

(*a*) *Drainage.*

Under conditions prevailing in Lancashire it would appear that adequate drainage might tend to encourage slug development and consequently increase the slug population. Improvements in the system of drainage allow the slugs greater freedom of movement and a wider range of activity. The raising of the soil temperature at certain times of the year would enable feeding to continue for a longer period and the eggs to hatch more quickly, and would probably induce a higher survival rate among the young slugs. The improvement in plant growth would result in an increased food supply for the slugs and enable the crops to support a more extensive slug population. The high degree of humidity in the atmosphere and the heavy rainfall which is well distributed throughout the year, together with the general prevalence of heavy soils, make it practically impossible to limit the soil moisture to such an extent that slug development is hindered.

(*b*) *Manuring.*

Examination of slug-infested soils in east Lancashire suggests that there is a fairly close relationship between the amount of organic matter present in the soil and the extent of the slug population. The application of organic matter to the soil increases its water-holding capacity, thus rendering it more favourable for slug development. It also provides

undecomposed humic material on which the slugs feed. This favourable influence of organic matter has already been noted by Morris (13), who has shown that at Rothamsted the application of farmyard manure to land previously unmanured increased the number of Pulmonates present from 13,500 per acre to 33,700 in the course of a year, an increase of about 150 per cent.

In the areas of east and south Lancashire where slug infestation is most serious organic manures are used almost exclusively on the holdings. A consideration of conditions suggests that the substitution of dressings of artificial manures for the usual organic dressings would tend to modify soil conditions and render them less suitable for slug development. No figures are available to support this suggestion, but on certain allotments in Manchester enterprising gardeners have ceased using organic dressings and substituted dressings of artificial manures. These men state that the amount of slug injury on their holdings has been greatly reduced though slug injury is still persistent on adjoining allotments.

(*c*) *Cropping.*

Since all garden crops appear equally susceptible to slug attack crop rotation has little influence on slug intensity. Conditions are especially favourable for slugs where intensive culture is followed, since the land is scarcely ever free from crops and large amounts of crop residue are continually being ploughed in. Other details such as the presence of compost heaps, the disposal of refuse and crop residues and the general hygiene influence the intensity of slug infestation under market-garden conditions.

Under ordinary farming conditions the system of crop rotation probably aids in limiting slug development. While a crop is present on the land conditions are favourable for slug development, but during the absence of a crop, particularly when the land is bare fallowed, the slugs are compelled to depend on organic matter in the soil for food. As this is exhausted conditions become less favourable for the slugs and their development is checked. This fluctuation in conditions probably maintains the pest in a more or less static condition and keeps the amount of injury within reasonable limits. Heavy losses from slug attack on general farm crops are reported only when unusually favourable conditions have permitted the slug population to increase far above normal, or when adverse conditions have so checked the growth of the crops that the normal slug depredations, which pass unobserved at other times, are readily apparent.

4. *Slug movements.*

The daily movement of slugs is widely recognised. In the evening the slugs leave their shelter and come out on the surface of the soil to feed. In the morning there is a return to suitable shelter. During mild wet weather this daily movement is somewhat obscured by numbers of slugs feeding continuously throughout the day. About June, provided that conditions of temperature and moisture are suitable, surface feeding reaches a maximum, since at that time there is usually an abundance of young succulent growth. In cold weather feeding at the surface is reduced to a minimum, the slugs generally remaining deep in the soil feeding upon roots and tubers and organic matter.

Migration from one locality to another seems to take place under certain conditions, such as when cultivated areas adjoin woodland or grassland. It is difficult to determine whether these movements are a direct response to the attraction of food during the summer months, and that similar movements towards shelter take place in the opposite direction in the autumn, or if it is a definite migration to a more favourable locality. Possibly both factors are concerned. The progressive dispersal of slugs from areas of high infestation, as these become over-populated or cease to be favourable from other causes, would tend to have a balancing effect and maintain the slug population of a locality at practically the same level all the time.

5. *Natural enemies.*

The slug appears to be singularly free from natural enemies. Birds, which perhaps exert the greatest influence on the slug population, do not seem to devour slugs to any great extent. In the studies (18) carried out from 1903 to 1914, first under the auspices of the Board of Agriculture and later in conjunction with the British Association for the Advancement of Science, it was apparent that save in one or two instances, slugs formed no main part of the diet of the species of birds under examination, but as a rule occurred only casually. Slugs were found in the crops of rooks and starlings, and also in those of jackdaws, thrushes, blackbirds and field-fares, but in no case to an outstanding degree. Subsequently Collinge's observations (5) revealed that slugs and snails formed 6·5 per cent. of the animal food of starlings, but as a limiting factor even this is scarcely appreciable. Slugs have also been found in the crops of pheasants and partridges, and C. P. May, M.A., of the Lancashire Agricultural Staff (*in litt.*), has found them in the crop of a wood pigeon. In view of the range of bird species which are known to attack slugs, it

seems likely that the nocturnal habits of slugs account for the fact that they do not form a more considerable part of the dietary of birds. Slugs are devoured by moles, toads and shrews, and carnivorous beetles of the families Carabidae, Staphylinidae and Lampyridae are also stated to attack them[1].

V. Conclusions.

From a study of slugs in relation to their environment certain facts become apparent, the most important being that moisture and food supply exert the greatest influence on slug development. Soils with a high water-holding capacity are likely to be infested with slugs, and where the use of organic manures and the development of intensive culture afford an abundant food supply slugs are also numerous. Where these factors occur simultaneously, as in large portions of Lancashire and north Cheshire, slugs become a primary pest.

The presence of organic matter in the soil facilitates the development of young slugs and accelerates the maturing and reproduction of the older slugs by providing food during the winter period when crop plants are less readily available. The practice, common in east and south Lancashire, of using an abundance of undecayed organic matter such as fallen leaves, undecomposed household and garden refuse, street sweepings, etc., to lighten the soil, increases the amount of organic matter in the soil and is invariably accompanied by heavy slug infestation. The substitution of dressings of artificial manures for the customary dressings of organic manures is likely to do much to reduce slug infestation, since soil conditions would be made less favourable for the development of slugs.

Field observations indicate that in Lancashire and north Cheshire the lime status of the soil makes little difference to the extent of slug injury, only extensive areas of raw peat being fairly free from slugs. Improvement in general soil conditions in response to the demand for more satisfactory conditions for plant growth seems likely to increase also the suitability of the soil for slug development, particularly since such improvement is usually followed by more intensive cropping. Natural enemies appear to have practically no modifying influence on the slug population.

The study of the slug population of a limited area, as indicated by the numbers of slugs collected from traps, seems to reveal the difficulties in the way of dealing with the slug problem. It would suggest that localised efforts aimed at the destruction of slugs have little value, since

[1] Theobald (1905), *Journ. Board of Agriculture*, XI, 650.

the slug population of the garden concerned seemed unaffected after several years of persistent trapping. Direct measures against slugs can only be regarded as temporary, but may be of value for protecting individual crops during the period of growth when they are most susceptible to attack. Direct measures of control usually involve the use of substances lethal or repellent in their effects, and owing to the tendency of the slug population of an area to be stable it would appear that repellent substances might prove more efficient for crop protection than lethal substances.

The density of the slug population seemed readily influenced by changes in weather conditions, favourable weather conditions during a season causing a marked increase in the numbers collected from the traps and unfavourable weather conditions producing a corresponding reduction. This would indicate that measures to control slugs must aim at producing changes in the slug environment and so render it less suitable for the development of slugs. In Lancashire and north Cheshire it seems that the direction of environmental changes would lie in the modification of the soil so as to reduce its water-holding capacity where this can be done without deleterious effects on the crops, and the reduction of the organic matter in the soil through the substitution of inorganic manures for the organic manures in present use.

PART II. EXPERIMENTS ON THE CONTROL OF SLUGS.

I. Introduction.

A considerable amount of work has been done from time to time on the control of slugs and numerous substances have been recommended as giving a good measure of control. The crepuscular and nocturnal habits of slugs have, however, rendered the field experimental work on control difficult, since the application of materials which aimed at killing the slugs by contact had to be made at night.

In 1913, at Harper Adams Agricultural College[1], tests on mixtures of lime and salt indicated that several dressings applied at intervals during the night yielded some measure of control, but the method was laborious and rather uncertain in its results.

In 1925, Anderson and Taylor (1), of Leeds University, conducted experiments on the control of slugs damaging field crops. They first tested materials in the laboratory and, selecting mixtures of copper sulphate and kainit for further trial, proceeded to test under field conditions the value of a single dressing during the night. Using dressings of 4–6 lb.

[1] *Harper Adams Agricultural College Annual Report* 1913.

copper sulphate in 1 cwt. kainit at the rate of 2–3 cwt. per acre, they obtained promising results on crops of oats, sugar beet and peas.

Investigating the value of both poison bait and substances which killed by contact under laboratory conditions, Hodson (8) found that though some good might accrue from the use of bran poisoned with paris green, an irritant like aluminium sulphate in a saturated solution of lime in water seemed more likely to prove efficient. Working later under field conditions, Hodson (9) found that aluminium sulphate both in solution and as a powder mixed with twice its weight of lime proved satisfactory, but that a bait of bran and paris green, 1 in 20 by weight, was more suitable for the treatment of large areas.

In the tests which were carried out at Manchester the species of slug used was *Milax sowerbii* Fér., since it was most common in the district. In the investigations carried out by Hodson the species of slug used was *Agriolimax agrestis* L., and it is probable that some of the differences in slug reaction were due to the use in experiments of a different slug species. Hodson, for instance, found aluminium sulphate lethal to *A. agrestis*, but when this material was applied to *M. sowerbii* it was easily cast off with the slime.

II. Laboratory experiments.

1. *Tests with control substances.*

Simple laboratory tests were carried out to obtain information which would serve as a guide in selecting materials for use in field experiments. A number of materials were used; some already known to be lethal to slugs, and others chosen for their cheapness and ready availability. In this connection the writers are indebted to Mr R. H. Clayton, Director of Manchester Oxide Co., for furnishing a number of the materials used. The tests were made on *Milax sowerbii*, as this species was most easily obtained.

In the first series of tests the slugs were lightly dusted with the chemicals and their reaction noted. In Table I the substances are grouped in accordance with their effects on the slugs.

All the substances in Group 1 were lethal to slugs in a reasonably short time, drained creosote salts and ammonium sulphate acting rather less rapidly than the others. Most of the substances were easy to handle, but the sulpho-cyanides were so deliquescent that it was felt they would be useless for large-scale trials. The substances listed in Group 2 were not lethal to slugs when lightly dusted over them. The slugs readily threw

off the chemicals with the slime and appeared to suffer no ill effects after one application.

Table I.

Group 1. Lethal to slugs.

Chemical	Remarks
Copper sulphate	Slugs died almost immediately
Calcium cyanide	,, ,,
Calcium carbide	,, ,,
Sodium carbonate (washing soda) ...	,, ,,
Corrosive sublimate	,, ,,
Sodium sulpho-cyanide	,, ,,
Ammonium sulpho-cyanide	,, ,,
Potassium sulpho-cyanide	,, ,,
Barium sulpho-cyanide	,, ,,
Drained creosote salts	Slugs died in 5–10 minutes
Ammonium sulphate	,, ,,

Group 2. Not lethal to slugs.

Chemical	Remarks
Copper carbonate	Easily cast off with slime
Aluminium sulphate	,, ,,
Flowers of sulphur	,, ,,
Green sulphur	,, ,,
Copper sulpho-cyanide	,, ,,
Lead sulpho-cyanide	,, ,,
Sodium nitrate	,, ,,
Thio-urea	,, ,,
Used calcium carbide	,, ,,
Precipitated chalk and creosote ...	,, ,,
Flake naphthalene	,, ,,
Precipitated chalk and chlor-cresylic acid	,, ,,
Potassium permanganate	,, ,,
Ammonium chloride	,, ,,

2. *Tests with lethal substances mixed with soil.*

The substances in Group 1 of Table I were each mixed with soil to give a series of concentrations approximating to those which would obtain if amounts of from 1 to 6 cwt. were applied per acre. Slugs were then introduced into the various mixtures and the results recorded after twenty-four hours' exposure. At the 1 cwt. per acre concentration only calcium cyanide proved lethal to the slugs. With copper sulphate the concentrations of 4 cwt. per acre and upwards proved lethal. When mixed with soil at varying rates up to 6 cwt. per acre none of the remaining substances proved efficient against *Milax sowerbii.*

3. *Tests for repellent effects.*

A range of substances was next tested for their value as repellents, by placing the slugs within a circle of each. With most of the substances the slugs merely passed through and showed no reaction, but in the case of sodium carbonate (washing soda), aluminium sulphate, ammonium sulphate, creosote in precipitated chalk, drained creosote salts and

copper sulphate, the slugs avoided the ring of chemicals and made no attempt to pass through.

Potassium permanganate, mercuric bichloride, ammonium chloride and phenol were made into solutions and mixed with soil. Slugs were placed within rings of the treated soil and the behaviour noted. A definite repellent action was noted in the case mercuric chloride and phenol when used at a strength of 1 in 500. With further dilution phenol rapidly decreased in efficiency, but the mercuric bichloride retained its efficiency at a strength of 1 in 1000.

From the preliminary tests it was apparent that several substances were definitely lethal to slugs. When considering efficiency in relation to cost, availability and ease of handling, copper sulphate, washing soda and creosote salts appeared most suitable for experiments on a field scale. Creosote in precipitated chalk and corrosive sublimate in solution seemed to have some value as repellents, therefore they were included among the substances selected for further tests.

III. Field trials, 1928–29.

Field tests were designed to determine the value of the selected substances under field conditions, and cabbage plants were used as indicators because of their comparatively high susceptibility to the attacks of slugs.

1. *Treatment of the soil before cropping.*

(*a*) *Preliminary tests.*

(i) *Copper sulphate at* 1 *cwt. per acre.* Copper sulphate in ordinary crystalline form was applied to the soil at the rate of 1 cwt. per acre and appropriate untreated plots arranged as a check. Cabbage plants were set out about a week after the treatment. The plants were examined daily for the first fortnight, and afterwards at short intervals until maturity. The following results were obtained:

Table II.

Plot	Treatment	No. of plants lost through slugs	No. of plants reaching maturity	% loss through slug attack
1	Copper sulphate	51	117	30·4
2	Untreated	72	99	42·1
3	Copper sulphate	70	106	39·8
4	Untreated	87	100	46·6

The land used for these experiments had been bare fallowed for a year previous to the planting of the cabbages. Slug attack commenced within

twenty-four hours after planting and was intense for about three weeks. Attack was concentrated on the stem at or just below ground level, many of the plants being completely severed and others so nearly severed that they wilted and died. There was a noticeable absence of cabbage root fly, so that the figures were not complicated by the presence of other pests. The application of copper sulphate at the rate of 1 cwt. per acre seemed to have a beneficial effect, since the loss on the treated plots averaged 35·1 per cent., while on the untreated plots it was 44·3 per cent.

(ii) *To compare the effects of copper sulphate, washing soda and creosote in precipitated chalk as pre-cropping treatments.* The land was apportioned into eight plots to allow suitable check plots. The dressings were thoroughly worked into the surface soil and three weeks later the land was planted with cabbages. Daily examination was made during the first fortnight after planting, and afterwards the plants were examined every two or three days until five weeks after planting.

Table III.

Treatment	Plot	No. of plants	No. of plants destroyed by slugs	% destroyed for each treatment
Washing soda 4 cwt. per acre	1	24	17	
	1 *a*	24	19	75
Untreated	2	24	12	
	2 *a*	24	19	64·6
Copper sulphate 2 cwt. per acre	3	24	12	
	3 *a*	24	7	39·5
5 % creosote in precipitated chalk 5 cwt. per acre	4	24	10	
	4 *a*	24	12	45·8

In this experiment the value of copper sulphate was again apparent. Washing soda applied at the rate of 4 cwt. per acre appeared to have no beneficial influence three weeks after its application, the greatest losses from slug attack occurring on the washing-soda plots. The results following the use of 5 per cent. creosote in precipitated chalk compared very favourably with those following the use of copper sulphate.

The conclusion drawn from these preliminary tests was that some benefit might accrue from pre-cropping treatment with copper sulphate or 5 per cent. creosote, where the land was heavily infested with slugs. One advantage of copper sulphate is that it can be obtained ready for use, whereas the use of the chalk and creosote mixture entails the labour of mixing. On the other hand the creosote and chalk mixture will not increase the soil acidity, but the continued use of copper sulphate would tend to aggravate the acid soil conditions already widely prevalent. The

increase of soil acidity could be readily avoided if an equal quantity of lime were used wherever a dressing of copper sulphate had been applied.

(*b*) *Further trials with copper sulphate.*

Further trials with copper sulphate at the rate of 1–2 cwt. per acre were organised under allotment conditions in east Lancashire during the winter of 1927–28. Owing to unforeseen circumstances[1] numerical data could not be obtained for these experiments, and only the general impressions of the allotment holders and nurserymen were available. They were unanimous in affirming that loss from slug attack was considerably reduced as a result of the copper sulphate treatment.

It became apparent that owing to the movement of slugs from untreated to treated areas it would be exceedingly difficult to obtain numerical data which would indicate the value of pre-cropping treatment. When trials were organised in the winter of 1928–29, the experimental plots were separated by trenches in which copper sulphate was frequently scattered. Save in one instance, no data could be obtained from these experiments, for the weather conditions prevailing in the spring of 1929 so checked slug development that no injury was noted on either treated or untreated plots until well into the summer after the crops had passed the susceptible stages. Similar conditions were observed on farm land which had been treated with copper sulphate to prevent slug attack on oats.

In one instance where continuous observations were made on a piece of garden ground, some figures indicating the results of the pre-cropping treatment with copper sulphate were obtained, though slug injury was much less intense than usually experienced. The land was treated at the rate of 2 cwt. copper sulphate per acre on diagonally opposite quarters, with trenches between the treated and untreated plots. After an interval it was planted with potatoes, runner beans and cabbage, all highly susceptible to slug attack. The following are the details of the experiment and the results obtained.

Table IV.

Date of treatment	Date of planting	Crop	Total no. of plants	No. injured by slugs	% injured
26. vi. 29	3. vii. 29	Potatoes	575 tubers	64	11·1
Untreated	,,	,,	640 ,,	126	19·6
26. vi. 29	,,	Beans	200 plants	4	2
Untreated	,,	,,	200 ,,	6	3
26. vi. 29	,,	Cabbage	40 ,,	1	2·5
Untreated	,,	,,	40 ,,	3	7·5

[1] A period of three months elapsing between the departure of Mr Wood to undertake other work and the arrival of Mr Thomas to continue the field work on slugs.

It will be seen from these figures that although the injury caused by slugs is slight, there are indications of the value of pre-cropping treatment with copper sulphate.

2. *Tests of repellents.*

(*a*) In order to test the value of various substances and mixtures as slug repellents, land set out with cabbage plants was marked off in double rows, two rows containing forty plants per row constituting a plot. The treatments were duplicated in order to balance the effects of a possible unevenness in distribution. Corrosive sublimate solution (1 in 1000) was used at the rate of $\frac{1}{4}$ pint per plant. 1 per cent. mixtures of creosote in precipitated chalk and chlor-cresylic acid in precipitated chalk, and also naphthalene (grade 16) were used at the rate of $\frac{1}{4}$ oz. per plant and applied evenly round the stem the day after setting out. Daily observations were made while slug attack was imminent, and the following table gives the results of the treatments.

Table V.

Treatment	No. set out	No. destroyed by slugs	% destroyed by slugs
Chlor-cresylic acid in chalk ...	160	139	86·8
Creosote in precipitated chalk	160	73	45·6
Naphthalene	160	87	54·3
Corrosive sublimate	160	38	23·7
Untreated	160	109	68·1

From these results it was apparent that corrosive sublimate was highly efficient as a repellent, and that a 1 per cent. mixture of creosote in precipitated chalk might be of value under conditions where the use of a solution was impracticable.

(*b*) In order to test further the value of dry dressings a second series of trials were carried out. The substances used were copper sulphate in powder form, the creosote and precipitated chalk mixture, and also nitrate of soda and sulphate of ammonia which have a stimulating influence on the plant and might enable it to develop quickly beyond the susceptible stage. Two rows of twenty plants each constituted a plot and the series of plots were duplicated.

Table VI.

Treatment	No. of plants	No. destroyed by slugs	% destroyed
Untreated	80	36	45·0
Nitrate of soda 3 gm. per plant	80	43	53·7
Copper sulphate 3 gm. per plant	80	31	38·7
Sulphate of ammonia 3 gm. per plant ...	80	39	48·7
Creosote in precipitated chalk 7 gm. per plant	80	12	15·0

This experiment gave further evidence of the value of creosote in precipitated chalk as a repellent. Observations indicated that it was not advisable to use undiluted copper sulphate in such close proximity to the plants, because when slight injury by slugs occurred the copper salt was absorbed by the wounded surface and the plant died in a few days. Nitrate of soda and sulphate of ammonia appeared to give no protection to the plants but stimulated the growth of those which escaped attack. These substances can be of value, however, in accelerating growth and pushing the plant through the susceptible period.

(*c*) A further trial of repellents was carried out on land which had been treated with copper sulphate prior to the setting out of the cabbage plants, and the following results obtained.

Table VII.

Treatment	No. of plants	No. destroyed by slugs	% destroyed
Check—copper sulphate (pre-cropping) ...	80	23	28·7
Nitrate of soda 3 gm. per plant	80	34	42·5
Copper sulphate 3 gm. per plant	80	20	25·0
Sulphate of ammonia 3 gm. per plant ...	80	28	35·0
Creosote in precipitated chalk 7 gm. per plant	80	11	13·7

Although there is a general reduction in the percentage of plants lost through slug attack it appears that the pre-cropping treatment has only a slight effect and that the main influence is that of the repellent used after the crop is set out. The value of the creosote and chalk mixture is again outstanding, thus affording definite verification of the results of the two preceding experiments.

In a similar manner corrosive sublimate was tested with pre-cropping treatments. Corrosive sublimate was used in solution at a strength of 1 in 1000 and applied at the rate of $\frac{1}{4}$ pint per plant.

Table VIII.

Treatment	No. of plants	No. destroyed by slugs	% destroyed
Check—copper sulphate (pre-cropping)	48	31	64·5
Corrosive sublimate	48	7	14·5
Copper sulphate (pre-cropping) and corrosive sublimate ...	48	1	2·0
Creosote and chalk (pre-cropping) and corrosive sublimate	48	2	4·1
Washing soda (pre-cropping) and corrosive sublimate ...	48	1	2·0

This again indicates that the effect of the repellent used after the crop was set out was the most important factor reducing slug attack.

(*d*) Further trials along the lines of the preceding trials were organised in the spring of 1929, but owing to the absence of slugs no results could be obtained.

3. *Tests with paris green.*

Experiments with paris green and bran bait were carried out in east Lancashire in 1926–27. The bait consisted of 1 lb. of paris green added to 30 lb. of bran and moistened with water to which a little treacle had been added. This amount was sufficient for one acre. In some cases the bait was broadcast on the surface of the soil in the evening, and in others it was lightly worked into the surface of the soil in an endeavour to lengthen the period of its efficiency.

In all some ten acres of land were treated. Although appropriate control plots were left and frequent examinations conducted, the difficulties of estimating the results were so great that only a general impression of the value of the treatment can be recorded. In most instances the bait seemed to afford the plants some protection for about a fortnight. After that period the bran seemed no longer attractive to the slugs, probably owing to the development of moulds. Though some dead slugs were found on the treated plots, their numbers were insignificant, and it was apparent that some indicator other than the number of dead slugs was necessary to express the value of the treatment. The influence on crop plants was considered, but this proved unsatisfactory as there was a wide range of plants of varying degrees of susceptibility to slug attack; and many of the crops, as for instance carrots and potatoes, could not be carefully examined before and after the application of the bait, since the injury by slugs was not generally apparent until harvest. In one case a system of trapping was carried out on treated and untreated plots in order to ascertain the possible influence of the bait on the slug population. Tiles were used as traps and were placed one to each 10 square yards of ground. The bait was applied at the beginning of June and trapping was carried out until July 20th. The following figures were obtained.

Table IX.

Treatment	Slugs per 100 sq. yds.
Paris green and bran bait	146
Untreated check	213

These figures indicated an appreciable reduction in the number of slugs on the treated area, but the probability of error due to the movement of slugs is recognised.

IV. Discussion of results.

The difficulties of testing out direct measures for slug control are very considerable, and only after some years' work has it been possible to record information which might be considered reliable. Even yet much of this must still be regarded as general impressions gained from the study of the slug problem in the field.

From field trials it is apparent that the use of certain substances will give some measure of protection against slug attack. Copper sulphate, washing soda and creosote are definitely lethal to slugs, and when applied to the soil appear to destroy the slugs which come in direct contact with them. The value of these substances seems to depend upon the nature of the soil, the fineness of division of the chemicals, and their even distribution throughout the surface soil. The lethal power of the materials probably lasts several days, after which the slugs can travel over or through the soil without any apparent inconvenience.

The benefits derived from the application of these materials to the soil prior to the setting out of the plants were well marked. They appeared, however, of a temporary nature, and the treated areas seemed rapidly re-populated by slugs from adjoining areas. This tendency towards the maintenance of a stable slug population, which seemed to be indicated where trapping had been carried out persistently over a period of several years (p. 380), reduced the efficacy of the pre-cropping treatment, though the initial check to slug activity gave susceptible plants a chance to become established.

The use of copper sulphate broadcast over the ground in the presence of a crop is worthy of consideration, but because of the risk of injury to the crop its application needs care. Anderson and Taylor (1) recommend the use of a mixture of 6 lb. of copper sulphate and 1 cwt. of kainit used at the rate of 3 cwt. per acre. This should prove of value on a field scale, but the writers are of the opinion that under the conditions prevailing in the north-west of England the proportion of copper sulphate might be greatly increased. In a similar manner dry Bordeaux mixture (12), consisting of lime and copper sulphate and obtainable ready mixed, can be used where crops are suffering extensive injury from slug attack. Shallow trenches in which copper sulphate or a mixture of copper sulphate and ground limestone has been sprinkled will be found satisfactory for preventing the migration of slugs from hedge sides, ditch sides and waste ground to land which has been treated.

The use against slugs of bran bait poisoned with paris green needs further investigation in the north-west before its value can be definitely assessed, but from the results so far obtained the method does not seem as effective as others which were tried. Under conditions of high humidity the bran speedily loses its attraction, so that several dressings would be necessary to prevent the slugs returning to the crop plants.

During the course of these investigations the most satisfactory method of dealing with the slug pest appeared to be by the use of repellents. Experiments so far conducted have shown the value of corrosive sublimate 1 in 1000 and creosote and precipitated chalk 1 in 100, and further work will probably extend the list of substances suitable for this purpose. Under market garden and allotment conditions, where water is generally available, corrosive sublimate is to be recommended. A method of application which appears speedy and efficient has been described by Britton (4), and it should be possible to adapt a knapsack sprayer to apply the solution on a fairly large scale. Under field conditions where water is less easily obtained, the creosote and chalk mixture should prove satisfactory and could be applied by means of a powder sprayer. It might be found possible to use the chalk and creosote in the form of a thin paste, into which bundles of such plants as cabbage and cauliflowers might be dipped before planting out, thus reducing the time and labour involved. This method, however, has not been investigated.

Since the slug problem in the north-west of England is closely bound up with conditions of soil and climate, the control measures which have been described can only be regarded as giving temporary relief. Even where such measures are carried out persistently there seems little likelihood that any marked influence on the slug population will accrue. Any considerable relief can only follow alteration or modification in the character of the soil so that conditions are no longer suitable for the maintenance of a large slug population. It seems likely that in the north-west of England this change in soil conditions may be produced by alteration in the system of manuring, for allotment holders who have substituted artificial manures for organic dressings have observed a remarkable reduction in loss from slug attack, although on adjoining allotments receiving the usual quantities of organic manure without artificials losses from slug attack were very severe.

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EXPERIMENTS ON POTATO SICKNESS

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(With Plate XXIV.)

Since the experiments on potato sickness carried out with potted plants in 1928–29, and published elsewhere (1), the work has been carried further and the results are put on record here. It may be pointed out that in 1928, the first year of inoculation, no difference was observed between the potato plants grown in sterilised (autoclaved) soil (control) and the series inoculated with *Corticium Solani*; a very slight difference, hardly significant, was noticeable between those in the sterilised soil and (*a*) those infected with the eelworm *Heterodera schachtii*, and (*b*) those infected with both the eelworm and the fungus; but there was a marked difference between the plants grown in sterilised compared with those in unsterilised soil.

These series of inoculated and uninoculated plants died down, the haulm and tubers were carefully removed, and the pots of soil kept under outdoor conditions throughout the winter 1928–29. They were again planted and additional inoculations given in the following spring.

In 1929, a big difference in growth was observed between the sterilised and the eelworm plus fungus series, and a significant difference noted between the sterilised and (*a*) the eelworm, (*b*) the unsterilised, and (*c*) the fungus series, although with (*c*) there was a grow away inclination as the plants "tended to pick up towards the end of June, and then in marked contrast to the eelworm series three continued to grow and retain a perfectly healthy colour in the second week of July, despite the fact that the *Corticium* stage of the fungus was abundant at the base of the stems."

Whatever other conclusions could be drawn from these experiments, it was evident that the cause of potato sickness was a biological one, since the disease was remedied by steam-sterilising the affected soil.

That the inoculated plants did not show marked symptoms the first year, and that the pots of soil were kept under outdoor conditions throughout, invites the criticism that another biological factor had crept in for the second year.

Of the possible factors, the fungus *Colletotrichum atramentarium* suggested itself, since it is always present on dying potato plants. Abroad, in South Africa and Canada, it is the cause of a recognised potato disease, and in this country it has been recorded by Salmon and Ware (2) in association with *Heterodera schachtii* on potatoes. On the other hand preliminary pot experiments conducted in 1929 (3) showed that normal potato plants were produced by planting potato tubers naturally infected (4) with the sclerotial bodies of *Colletotrichum atramentarium*—contrary to the results of Dickson (5) (Canada).

In 1930, more pot experiments were carried out to ascertain if *Colletotrichum atramentarium* was a factor in producing the extreme symptom recorded in the 1929 experiments on potato sickness.

The work was run on similar lines to the preceding experiments, the soil was obtained from very bad potato-sick land, and that required for inoculation purposes was submitted to autoclaving. The pots were previously soaked in very weak formalin. No manure was applied to the soil. The Eclipse potatoes used for "seed" were steeped in mercuric chloride solution. The pots were not plunged in soil but kept on an asphalt pavement and liberally watered. Since only a comparatively small number of eelworm cysts were available the inoculations were made directly around the "seed" tubers.

The following series, each consisting of four pots, were set up on April 15th:

Series 1. Inoculated with *Heterodera schachtii* cysts and *Colletotrichum atramentarium*.

Series 2. Inoculated with *Heterodera schachtii* cysts, *Corticium Solani* and *Colletotrichum atramentarium*.

Series 3. Control, sterilised soil. No inoculation.

Series 4. Inoculated with *Colletotrichum atramentarium*.

Series 5. Inoculated with *Colletotrichum atramentarium* and *Corticium Solani*.

The marked difference in growth rates that were obtained in 1929 (second year) were not observed with the 1930 series (first year), but the effects of the inoculations in Series 1 and 2 compared with Series 3, the control, were most certainly large enough to be significant on June 27th (Plate XXIV, figs. 1 and 2). The slightly retarded effects in Series 5, or the variation between Series 1 and 2, observable in the photograph were not enough to be significant.

Following on from June 27th, all the retarded plants appeared to

Fig. 1.

Fig. 2.

CHEAL.—Experiments on Potato Sickness (pp. 401-403).

make up leeway, and the difference in growth compared with the control series became much less apparent.

It may be recorded here that a repetition in 1930 of the 1929 experiments with the pot culture of King Edward potatoes from "seed" naturally infected with *Colletotrichum atramentarium*, both in autoclaved and in natural soil, fully confirmed the 1929 results. Normal plants were produced from naturally infected tubers.

Summary.

Following results already published of the effect of inoculations on potato plants with *Heterodera schachtii* and *Corticium Solani* in 1928 and 1929, further pot experiments were carried out in 1930 with an additional organism *Colletotrichum atramentarium*. The results showed a definite effect, the first year of inoculation, in those pots where *Heterodera schachtii* was present.

There was not enough evidence to support the view that the absence of *Colletotrichum atramentarium* in the 1928 inoculations accounted for the slight symptoms of potato sickness produced that year compared with results obtained in 1929 (the second year).

I wish to acknowledge the facilities provided at the Kirton Agricultural Institute, and am again much indebted to Prof. V. H. Blackman, Imperial College of Science, for his helpful criticism.

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(1) 1928 *Journal of Ministry of Agriculture*, June, 1929.
(1) 1929 *Gardeners' Chronicle*, August, 1929.
(2) *Journal South Eastern Agricultural College, Wye*, 1929, pp. 165 and 167.
(3) *Gardeners' Chronicle*, December, 1929.
(4) *Gardeners' Chronicle*, December, 1928.
(5) *Trans. Roy. Soc. Canada*, XVII, 1923.

EXPLANATION OF PLATE XXIV

1. Inoculated with *Heterodera schachtii* and *Colletotrichum atramentarium*.
2. Inoculated with *Heterodera schachtii*, *Colletotrichum atramentarium* and *Corticium Solani*.
3. Control. Autoclaved soil. No inoculations.
4. Inoculated with *Corticium Solani*.
5. Inoculated with *Corticium Solani* and *Colletotrichum atramentarium*.

(*Received January 27th*, 1931.)

PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS. I

Annual General Meeting of the Association held at 11.30 a.m. on Friday, February 20th, in the Botanical Lecture Theatre of the Imperial College of Science and Technology, London. The Chair was taken by the President, Dr A. D. Imms, F.R.S.

After the business the President opened a discussion on "Biological Races and their Significance in Evolution" in which the following papers were read:

I. Biological Races in Insects. By W. H. Thorpe, M.A., Ph.D., of the Imperial Institute of Entomology.

II. Biological Races in Nematodes. By T. Goodey, D.Sc., of the Institute of Agricultural Parasitology.

III. Biological Races in Fungi. By Wm B. Brierley, D.Sc. of the Rothamsted Experimental Station.

IV. Biological Races in Bacteria. By P. Bruce White, B.Sc., of the National Institute for Medical Research.

V. Biological Races in Seed-bearing Plants. By W. B. Turrill, D.Sc., of the Royal Botanic Gardens, Kew.

OPENING REMARKS BY THE PRESIDENT.

In opening the subject, which forms to-day's discussion, I intend to make only a few preliminary remarks and will not intervene between you and the leading speakers for more than a few minutes.

Intra-specific races have long been known to occur among most of the great groups of living organisms. Since the individuals of these races differ from typical members of the species to which they are referred, in biological or physiological characters, they constitute what are commonly termed biological races. The main features of such races are differences in habit or behaviour: morphological differences may or may not occur and, in any case, they are generally slight in character. For purposes of the present symposium our subject may be discussed from three points of view. (1) Evidence of the existence of such races, (2) their significance in relation to evolutionary problems, and (3) their significance in relation to applied biology.

In the majority of cases biological races appear to be particular developments of trophic behaviour. Among insects, for example, they are very commonly phytophagic races. That is they betray an evident tendency to reproduce upon the same plant

species as they themselves have developed. At the same time such individuals may show correlated differences from typical forms both in size and coloration. From the evolutionary standpoint it is tempting to regard such races as the beginnings of species in the making. I hope evidence both for and against this point of view will be discussed in the course of this meeting. Where the characters are sufficiently stereotyped, as it were, and are inherited from generation to generation we soon find ourselves up against the enigma of what is a species. The most valuable data that can be brought forward at this meeting are records of actual experiments. Entomological evidence is not strong in this respect, whereas the mycologists are in a much more secure position.

Reviewing the subject from the entomological standpoint certain experiments have been carried out whereby it has been possible to induce some kinds of insects to feed upon an unusual food plant in the absence of their normal host. In the next generation the preference for the new food plant became so marked that the original host was refused. At first sight this looks like the inheritance of an induced character, in fact much of the data bearing upon biological races are most difficult to explain except on a Lamarckian basis. I admit that such a confession would be regarded in many quarters as rank blasphemy, and that entomologists have not brought forward sufficient evidence which at the present day would satisfy most critics.

Now, in case any of you may think that I am a thoroughgoing Lamarckian I am going to dispel the idea by means of an hypothesis which I think may contain an element of truth. The point I wish to make is this. Once an insect has fed upon a new food plant, from the youngest larva or nymph, onwards, it has become conditioned to that plant. This conditioning impresses itself upon the chemotropic behaviour of the female to the extent that she selects that same plant species for egg laying. Her progeny feed upon this same host and this may go on generation after generation. I maintain that nothing more than conditioned reflexes are involved and that the only inheritable character is the faculty for conditioning which seems to be present in many species. The food plant it would seem merely influences the nature of the conditioning that takes place.

It is the business of a Society like ours not to allow itself to be carried too far into fundamentalism at the expense of utilitarianism. The problem will, therefore, occur to many of us: What economic or practical significance is to be attached to biological races? Where the latter are clearly in evidence it matters very little whether they are, biologically, races, sub-species or what not. The main point I wish to emphasise, in this connection, is that knowledge respecting the behaviour of such races is of the utmost importance. In cases where such races affect economic plants we may have to apply differential treatment as rigidly as if they were separate species. I hope and believe that more than one member of this audience will be able to bring forward evidence in support of this contention. With these few preliminary remarks the subject of biological races is now open for discussion.

I. BIOLOGICAL RACES IN INSECTS AND THEIR SIGNIFICANCE IN EVOLUTION.

By W. H. Thorpe, M.A., Ph.D.
(*Imperial Institute of Entomology, Farnham Royal, Bucks.*)

The occurrence of what have been variously called biological, physiological or intra-specific races among insects has long been known. Recent work has, however, extended our knowledge of this subject to a great extent, and the close bearing that it has upon various problems of variation and evolution is becoming increasingly clear.

A proper understanding of the subject is obviously of enormous importance to the economic zoologist, but the point I wish to stress now is that applied entomology has a contribution of great importance to bring to the study of problems of more academic interest.

We can, for the moment, define a species as a group of individuals distinguished from all other groups by the common possession of certain structural characters. A biological race may then be said to exist where the individuals of a species can be divided into groups, occurring in the same locality and showing definite differences in biology, but with structural differences, either very slight and inconstant, or completely absent.

I do not, of course, wish to imply that species are morphological entities whereas races are biological. We merely accentuate the morphological characters of species for the sake of convenience in systematic work. In reality species are distinct both morphologically and biologically, but in some cases the morphological aspect is most in evidence, in some cases the biological. Thus the idea of "physiological" species in Bacteria, biological races in Arthropoda, etc., and the meaning of the word "species," as generally used, appear to be fundamentally the same—except in so far as the absence of sexual reproduction in the bacteria imparts a somewhat different meaning to the word "species."

This phenomenon of biological races crops up in a great variety of groups in the animal kingdom. Examples are known among the Protozoa. In many of the lower groups, such as the Porifera and Coelenterata, morphological characters are very slight and unreliable, whereas biological differences are of great importance. In the Mollusca, Cuénot has described three forms of the cuttle-fish, *Sepia officinalis*, without doubt one species morphologically and occurring in the same locality, but biologically very effectively isolated from one another by differences in the reproductive and migration periods.

Somewhat similar cases are known in certain fishes, and later in these discussions we shall hear of the phenomenon as it concerns nematodes and other parasitic worms.

As far as insects are concerned the idea was first put forward as long ago as 1864 by Walsh, an American worker, who supposed, from his observations on various wood-boring and plant-feeding beetles, that races of this type, attached to different food plants, must exist. Walsh's work appears to have attracted little attention at the time of publication, and was totally neglected till Craighead drew attention to it in 1923.

Walsh established two categories, phytophagic variety and phytophagic species, according to whether intercrossing takes place or not, and regarded the second category

as equal in value to a species distinguishable on structural grounds. This arrangement cannot now be followed, for sterility of hybrids is no longer considered an infallible test of the validity of a species and few, if any, biological races are so strongly established that crossing *cannot* take place, although under natural conditions it may be extremely rare. Moreover, if we use the word "species" we should logically have to give them a specific name.

But it would clearly be impossible, in the Insecta at least, to unite in one category groups of individuals distinguishable morphologically, on an equal footing with other groups identifiable only by means of prolonged breeding tests. Even if "biological races" and "morphological species" are of equal value from the evolutionary or genetical point of view, to include them both in one systematic category would cause endless confusion.

The best course in such cases appears to be to use a varietal name such as *Pediculus humanus* race *capitis* Nuttall. Simple trinomials without the designation "race" have been used, but these should strictly be reserved for geographical sub-species. When a biological race has been thoroughly investigated it is obviously desirable that it should have some designation, but at the same time distinction must be made between geographical and biological races.

The close analogy between biological and geographical races will be seen from the definitions given already, but, whereas the continuance of a geographical race is ensured by geographical isolation, the biological race is isolated by biological differences, such as food and egg-laying preferences, and by disinclination for cross-breeding. Indeed, in a fully established biological race in a plant-feeding insect, for instance, one finds that the larvae of each form have a well-marked preference for the food plant of that particular race; that the adults have a preference for egg laying on that plant, and that there is a definite tendency for members of the same race to mate together rather than with individuals of another race.

At this point it is convenient to mention the "host selection principle," as it has been called, which has a close bearing on the subject, in that it suggests a method whereby a large class of biological races may have been brought into being. A definition of the principle has been given by Hopkins (1917), in ignorance of Walsh's work. He says that "an insect species which breeds on two or more hosts, will prefer to continue to breed in the host to which it has become adapted." If this is so, then it follows that the species in question is to some extent, at least, differentiated into biological races.

It would, of course, be quite impossible, in the course of half an hour, to give an adequate account of even a tenth of the known instances of biological races; to attempt to do so would be a mere waste of time. Cases are known from practically all the main insect groups.

All that I can do is to describe a very few examples chosen as representative of the subject and then consider what exactly is their theoretical significance. Further information will be found in my review of the literature concerning this subject (1930).

It will be convenient for the purposes of this discussion to divide the examples into two main categories:

(*a*) Biological races occurring naturally.

(*b*) Biological races produced experimentally.

Taking first naturally occurring races we can for convenience again subdivide into (i) those concerning plant-feeding insects, and (ii) those in which parasitic insects are involved.

In the Orthoptera there is one very striking instance known, and without doubt a great many others await discovery. Fulton (1925), in a study of variation in the Snowy Tree Cricket (*Oecanthus niveus*), found that in Oregon there are two races which differ only in their habits. Race "A" inhabits trees and is practically identical with individuals of the same species from the eastern States, the eggs being placed singly in the bark of prune and apple. Race "B" is confined to bushes in Oregon, and the eggs are laid in compact rows in the *pith* of shrubs, such as loganberry and wild raspberry. The females of each form select plants for egg laying which best meet the requirements of their characteristic mode of oviposition, and this appears to be the main factor in determining the choice of environment. Study of a large series failed to reveal any pigmentary or morphological characters by which the two forms could be separated, but they can be distinguished in the field by very well-marked differences in song, race "A" having a frequency of notes almost twice as great as Race "B." Not only were races "A" and "B" constantly different in song but both were distinct from the eastern form. The characteristic song and oviposition habits remain fixed when adults of one form are confined to the normal environment of the other, and it is difficult, and in some cases impossible, to induce "A" to lay eggs on shrubs or "B" on trees. Experiments suggest that there is a mating repugnance between the two forms, but much more work is necessary before this can be considered definitely established. The same author describes similar races of *Nemobius fasciatus* in Iowa, inhabiting different ecological niches and quite indistinguishable morphologically, although easily separable by song; but this species was not studied in such detail.

Another case which may be described is one which I have myself investigated to some extent (1929 and 1930). This concerns the Small Ermine Moths, *Hyponomeuta padella*, a common species found chiefly on apple, hawthorn, and blackthorn, and not infrequently a serious pest in orchards. The main points of interest are as follows.

The ground colour of the forewings of the moths is very variable, all shades from dark grey to pure white being found. These colour forms appear to be correlated to a considerable extent with the food plants, the dark grey form being most frequent on hawthorn and blackthorn, whilst the pure white form is predominant on apple. Careful investigation has failed to reveal any constant structural differences between these forms. Larvae taken from one host plant would not willingly feed on any other, but could be forced by starvation to adopt the food of the other race. When this was once accomplished they might show an actual preference for their new food, although those larvae reared on a strange plant generally emerged as rather undersized and often infertile moths.

In addition there are one or two interesting biological differences between the two forms. The cocoons of the apple-fed larvae are generally composed of a rather dense white silk, and the pupae are usually placed together in neatly arranged rows or packets. The hawthorn and blackthorn-feeding larvae, on the other hand, as a rule only spin very flimsy silken cocoons through which the pupae can be easily seen, and these are very frequently scattered at random in the web, close packets being the exception. Again, a leaf-mining habit is usually present in the first larval stage of the apple feeder, but not in the case of the hawthorn form.

A series of experiments carried out over a period of two years showed that hawthorn-reared moths, when given a choice of either hawthorn and blackthorn, or apple, placed 79·3 per cent. of their eggs on the former plants and 20·7 per cent. on the latter. Conversely, moths reared on apple and given a similar choice laid 90·25 per cent. on that plant, and 9·75 per cent. on hawthorn and blackthorn.

Experiments carried out with hawthorn and blackthorn-reared moths, giving them a choice of these two food plants, indicated that the hawthorn-blackthorn race is subdivided in the same way. Thus, out of 825 eggs laid by the hawthorn-reared insects 18·8 per cent. were laid on blackthorn and 81·2 per cent. on hawthorn, whereas out of a total of 4293 eggs laid by the blackthorn form corresponding figures were 69 per cent. for the former plant and 31 per cent. for the latter.

Other tests in which equal numbers of typical males and females of the apple and hawthorn races were placed together in cages, all the moths of one race being marked so that they could be easily distinguished, showed that the number of like matings was roughly twice as great as the number of crosses, or in other words, that the attraction between like forms was about twice as strong as that between unlike.

Similar results to these were obtained by Cameron in his work on the two forms of the Anthomyid fly, *Pegomyia hyoscyami*. The fly attacks beet and mangolds, as well as other members of the Chenopodiacae and Solanaceae, the larvae mining in the leaves. Cameron's experiments revealed that there were at least two biological races within the species, one race confined to the order Chenopodiaceae, and the other to the Solanaceae, and that within these two families different preferences might be shown. As in *Hyponomeuta*, there are two colour forms associated with the host plant preferences.

As a final example in plant-feeding forms one may cite the recent work of Kinsey on the gall wasps of the genus *Cynips*. Several instances are quoted by this author in which while two races may be almost, if not quite, indistinguishable morphologically, the galls produced are totally dissimilar.

Among parasitic insects a case which has particular theoretical interest is that of the human louse. For more than one hundred years the lice infesting the clothing and those infesting the head had been regarded as distinct species. In 1919 Nuttall showed that the characters by which the two were distinguished were unreliable and that they could only be regarded as a single species, and previously it had been shown (Bacot, 1917) that the hybrids were healthy and fertile to the F_3 generation. It is interesting that the differences between the two forms are such as might be accounted for by the different environments. Thus there are slight structural and colour differences which seem to be correlated with the life of the body louse under clothing, and with the habit of taking large meals of blood at longer intervals. The biological differences are even more interesting. For instance *capitis* is more active at lower temperatures, it can climb more actively on hair, its fertility is somewhat lower under experimental conditions, and it prefers to lay eggs on hair, whereas *corporis* prefers to lay on cloth, and when laying on hair does so awkwardly.

Finally it was shown that it is possible by rearing *capitis* on the arm for three or four generations, to transform them into typical *corporis*. The most significant fact is that this transformation was not brought about suddenly, immediately upon transference to the new environment, but was a gradual process covering three or more generations.

It is tempting at this point to leave the class Insecta and consider some of the similar instances exemplified by the mange mites of the genera *Psoroptes* and *Sarcoptes*. Much work has been done, and it is not possible to do more here than mention the general conclusions towards which it seems to point. In this group we are more than ever dependent on the work of the systematist, but the most recent and the most exhaustive systematic work all points to the conclusion that the forms of a species, such as *Sarcoptes communis*, occurring on a variety of animal hosts cannot possibly be regarded as distinct species. Yet it is often difficult, and in some cases apparently impossible, to transfer mites from one host to another.

It is worthy of note that there is a good deal of evidence suggesting that the same phenomenon is widespread amongst the plant-feeding mites—particularly as regards the gall mites of the family Eriophyidae.

There is a large number of interesting cases in which biological differentiation is associated with geographical isolation. These instances are essentially geographical sub-species, in which the chief distinguishing characters are biological. A detailed discussion of these would perhaps be out of place here, but some of the most interesting cases concern parasitic insects and aphids—both of which sections will, no doubt, be discussed by subsequent speakers. I will restrict myself to brief mention of one case, which has come particularly under my notice.

The Red Scale, *Chrysomphalus aurantii*, of the Orient is indistinguishable structurally from that of California, but the Chalcid parasite, *Comperiella bifasciata*, which in the Orient attacks both *Chrysomphalus aurantii* and *C. aonidum* impartially, when introduced into California can flourish only on the latter. There appears to be no doubt that this is due to a physiological difference in the scale from the two regions, for Mr Compere tells me that while oviposition on *C. aurantii* will take place freely in California, the host always destroys the larvae by phagocytosis.

The difficulty in all such cases is that of deciding whether the effects observed are due to genetical differences or to the direct effect of environment.

We can now pass to the consideration of those cases, theoretically the most interesting of all, in which new biological races have been produced experimentally.

I do not propose to describe in detail the early experiments of Schroder, Marchal and Pictet, concerning the production of races of insects adapted to new host plants. They are already so widely known that it seems unnecessary to recount them, and unfortunately they were not done on a sufficiently large scale to permit of any very far-reaching generalisations being based upon them. It will be more profitable to turn at once to the recent work of Harrison on the gall-making sawfly, *Pontania salicis*.

In experiments, carried out over a period of six years, Harrison was able to produce a new biological race adapted to *Salix rubra* from a pre-existing *S. andersoniana* race. He considers that his experiments indicate that an acquired character, in this case a modified egg-laying instinct, is inherited. The method adopted was as follows.

A race of *Pontania salicis* attached to *Salix andersoniana* was transferred to an isolated patch of ground, where *S. rubra* was the only willow available, the plants there being quite free from any gall-making insects. The female sawflies began to lay eggs on this form, and although at first the mortality was very high some survived, and in four years' time there was a flourishing colony. Specimens of *S. andersoniana* were then planted in among the other willows, but during the three years in which observations were made none of the sawflies showed any disposition to return to this

plant. Harrison concludes that in other words the "acquired habit of oviposition" on the new species had become germinally fixed, and the sawfly race was "being forced along an evolutionary path away from the parent species."

These experiments are of very great interest, and provide perhaps the clearest and most satisfactory case known among insects, of the artificial production of a biological race. However, the interpretation put upon these results by Harrison in stating that the new habit was germinally fixed, seems open to question. He does not seem to have considered the possibility that his results may be due to some sort of "larval memory," the adult having a tendency to seek out for oviposition a plant of the kind upon which she had fed as a larva. Such an explanation is by no means impossible, indeed, it is suggested by the very words of Walsh's "host selection principle" in its original form. If such host plant changes *are* of any significance in initiating evolutionary divergence, the postulation of a "mnemonic" preference keeping the nascent race to its new host, perhaps for a long period, till germinal differences appear, seems difficult to dispense with. It would also be of great interest to know if mating preferences were developed along with the change in oviposition response. As has already been mentioned, they are known to occur in natural biological races, but it is hardly conceivable that these could be *immediately* developed as a direct result of food plant change. If, however, a preference for a particular plant was handed on to the adults from the larvae the individuals attached to one plant would, one might suppose, be more likely to mate together than with members of another host race merely because of their proximity; and it is not difficult to imagine how a mating preference might then in time be built up as a direct result of the food plant change.

Finally, there are one or two anomalous cases, which, although as yet little understood, are of quite unusual interest. Previous to 1916 it had been noticed by Quayle and others that in the Corona district of Southern California it was becoming increasingly difficult to kill Red Scale (*Chrysomphalus aurantii*), an introduced pest of Citrus, by the usual means of fumigation with HCN. At first this was put down to some defect in the fumigation procedure, but it soon became evident that this was not a satisfactory explanation, for by 1921 other areas in Southern California were involved, and the trouble appeared to be spreading gradually outwards from the centres where it was first observed. That the effect was due to the presence of a resistant form of the Scale itself, and not to the effects of local climatic conditions on the efficacy of the fumigation process, seems to have been shown conclusively by the work of Quayle (1922). Since that time the resistant area has steadily increased, and by 1928 included a considerable portion of the adjacent counties of Riverside, Orange, and Los Angeles. In these areas the dose of HCN now required to give satisfactory results is so large as to be injurious to the trees. A similar reaction is exhibited by the Black Scale (*Saissetia oleae*), which first showed signs of developing resistance in 1912, the resistant form now having spread through a large area of Los Angeles and San Bernardino counties.

The obvious explanation is, of course, that continued fumigation results in the artificial selection of more vigorous individuals, thus in due course building up a vigorous and resistant race. But this fails entirely to explain the situation in regard to the Red Scale. In this insect the resistant form crops up in a few isolated areas, from which it spreads gradually. Elsewhere, even though fumigation has been practised for as long a period, no resistant races are developed.

A great deal of work will have to be carried out before it is possible to give a satisfactory explanation. It should, however, be noted that a preliminary attempt by Boyce (1928) to produce resistant races of *Drosophila* and *Aphis* by fumigation was unsuccessful. While individuals were found to vary in their vigour so that fumigation appeared to produce a slight increase in resistance, yet work carried on over seven generations failed to give definite evidence of any cumulative effect.

Turning now to a consideration of the possible evolutionary significance of these various examples; are they, as so many workers have supposed, really species in the making?

It appears to me that there are two sets of conditions in which phenomena, such as changes in host preference, may be of evolutionary significance.

Take first the case of a polyphagous species, capable of feeding on a great variety of plants in a given area; individual females laying eggs *at random* on these plants. While of course each individual larva would probably be restricted to one species of food plant throughout its life, merely because the egg from which it emerged happened to have been placed on that plant, this would not increase the probability of its progeny being reared on that species. In such a case the development of preferences restricting certain groups of individuals to certain hosts might be of importance. Even supposing that such preferences are not germinally fixed, but are merely memory reactions, they could persist as such for a long time and they would probably provide physiological barriers—lines of cleavage, so to speak—in an otherwise homogeneous population, thus aiding the spread of new variants.

As has long been realised, the great difficulty in regarding mutations or any other inheritable variations produced in small numbers, as the starting point of a new species, is that of accounting for the spread of such throughout a population. If the differences which serve to distinguish one species from another were of a kind likely to be of immediate survival value, then the problem would be simpler, but this does not appear to be the case with the general run of specific characters, still less of varietal ones.

The importance of geographical isolation in fostering such variations is undoubted, but in many cases it is unlikely that this factor can come into play until a comparatively late stage in the process; the first steps in the spread of a new variant are little assisted by geographical barriers. If, however, there is some form of physiological isolation such as that provided by various small biological differences and by a repugnance for cross-mating, then the process seems much easier to comprehend.

The mode of action of physiological barriers such as these, in promoting the spread of a variation, is essentially the same as that of geographical ones, and of course the existence of physiological barriers of some sort has long been assumed owing to the obvious insufficiency of geographical isolation alone to account for observed effects. The discovery of the wide occurrence of these biological differences then points to the conclusion that they may be of very great evolutionary importance.

An insect such as we have just been discussing might be described as both *potentially* and *actually* polyphagous. Now let us take the case of an insect which is potentially capable of feeding on a variety of hosts, but which is actually restricted to one or two of them. If such a species, through some sudden environmental change, is forced to feed on some *new* food plant and develops a race there, then the probability that the event may be of evolutionary significance seems even more obvious. Such a new host form might be of immediate survival value in that it might enable the insect to occupy an otherwise vacant ecological niche, previously closed to it, and

thus colonise a large area. That the correlation of such important biological differences with minute morphological characteristics of no survival value may provide an explanation of the persistence of these latter has of course long been realised.

If then this *is* a method of evolution, how consistently does it operate in nature? Meyrick (1927), for instance, tends to restrict the application of the principle on the ground that many closely allied species have the same food. But on the other hand there is plenty of evidence that food habits are plastic. Instances of large-scale habit change among insects will occur to every entomologist; but viewing the matter from another standpoint it at once becomes obvious that rapid development of new races cannot be going on uniformly in nature, or there would be no specific stability at all. In reality there are many instances in which it seems certain that the feeding habits of a species or genus of insects must have remained constant over a very long period of time. Constancy of food habits might, of course, be explained on the assumption that great mortality is always caused at the commencement of a host plant change, and that under natural conditions the few survivors would have an extremely slender chance of perpetuating the newly formed habit. Nevertheless, were the food habits of all insects as easily modified as, say, those of *Pontania* appear to be, even such initial difficulties at establishment would hardly seem sufficient to account for the stability of species as a whole.

Another difficulty is that the stronger the tendency of a species to develop a host preference the more difficult it becomes for that species to adopt new hosts. If, however, as seems possible in many cases, the host preference is mainly upon the psychological plane, without any *physiological* inability to change to a new host, we may have an explanation of certain of these difficulties. Thus, under normal conditions, the insect would remain constant, but during periods of exceptional stress, due perhaps to climatic or other environmental changes, the psychological preference might be overcome, with the result that new host plants would be adopted and a new race thus developed.

Finally, can the origin of biological races be explained on any non-Lamarckian theory?

In the majority of instances of experimental production of biological races a Lamarckian explanation has been suggested, but in many of these cases, such as those described by Schroder and Pictet, in which the experiments were done on a small scale, there was a considerable initial mortality, so that it is impossible to rule out the hypothesis of selection of pre-existing variations from a mixed population. There are, however, one or two cases among the Nematodes about which I hope we shall hear from Dr Goodey, which seem to make a Lamarckian explanation almost inevitable.

Harrison regards his experiments with *Pontania* as definite evidence of a Lamarckian effect, but while this is certainly the most obvious explanation the possibility of a mnemonic theory has not been completely excluded.

In the work of Nuttall and others on the transformation of *Pediculus capitis* into *P. corporis* we come against the difficulty that the change did not appear suddenly in the first generation after the change in feeding methods, but developed gradually over at least four generations. Keilin and Nuttall (1919, p. 325) expressly state that lice, *P. capitis*, which had been reared by Bacot for two years in the laboratory, were *intermediate* in structural characters between typical *capitis* and *corporis*.

In conclusion, then, we may say that many of these experiments are easily explained on some form of Lamarckian theory, but extremely difficult to account for

on any other lines. It seems certain, however, that none of them has been on a sufficiently extensive scale to carry complete conviction. They do, however, suggest most profitable fields for further work of this nature and, taken together, they provide a quite considerable amount of circumstantial evidence for the theory.

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II. BIOLOGICAL RACES IN NEMATODES AND THEIR SIGNIFICANCE IN EVOLUTION.

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In considering biologic races in nematodes I shall deal as briefly as possible with three different species: (1) *Ascaris lumbricoides*, a parasite of man, pig and certain other domesticated and wild animals; (2) *Tylenchus dipsaci*, and (3) *Heterodera schachtii* which are both plant-parasitic nematodes.

(1) *Ascaris lumbricoides*. The evidence for the specific identity of worms of this species from man and pig is very strong. Thus, in addition to the gross and detailed morphological similarity, it has been found that the number of chromosomes in both is forty-eight and that the size and shape of the chromosomes and of the chromosome complex is the same in both (Bakker, 1921, Barker, 1923). Infection experiments of

a reciprocal character, *i.e.* human to pig and pig to human, by feeding with embryonated eggs, indicate the occurrence of biologic races, since in none of the experiments, which have been very carefully conducted, has it been possible to get the human ascaris to come to maturity in the pig nor, on the other hand, the pig ascaris to grow to maturity in human beings (Koino, 1922).

There is also strong epidemiological evidence in favour of the occurrence of biologic races of this parasite (Caldwell and Caldwell, 1926).

If the two strains were interchangeable and equally infective to man and pigs then it would naturally be expected that where there was a high infestation rate in the pig a correspondingly high infestation rate would be found among the pig-keeping community. Where this aspect of the question has received attention, in Trinidad in the work of the Rockefeller Hookworm Campaign, the evidence is all in favour of the two races being distinct. Payne, Ackert and Hartmann (1925) said in a recent paper on the subject: "Considering the freedom of range allotted to the young domestic pigs and the ample opportunity they have of ingesting embryonated eggs of the human *Ascaris*, and considering the eating habits of the children and their opportunity of swallowing embryonated eggs of pig *Ascaris*, the incidence of these two ascarids in Trinidad does not furnish any epidemiological evidence that the pig and man are reciprocal hosts for them." So much for *Ascaris lumbricoides*.

(2) *Tylenchus dipsaci*. This species was first described by Kühn in 1858 causing disease in the flower heads of the fuller's thistle, *Dipsacus fullonum*. Kühn's and Ritzema Bos's experimental researches, carried out round about the eighties of last century, showed that the parasite has a wide range of hosts. Bos's work (1888–92) resulted in his bringing under the one specific name, *Tylenchus devastatrix*, several forms from different hosts previously ascribed to different species of the genus. To-day it is known as a parasite of 135 different kinds of wild and cultivated plants.

There is good evidence, on the one hand, of the occurrence of unspecialised polyphagous races, and, on the other hand, of certain well-defined specialised biologic races highly adapted to certain host plants.

Recent work by Quanjer (1927) has revealed the occurrence in Holland of a polyphagous race which was found on three wild plants in pastures and which causes disease in potatoes. When tested for its power to attack other plants it was found to be capable of setting up characteristic disease symptoms in fifty-two different kinds of plants, twenty-eight of which were new to science as hosts of the parasite.

Fox Wilson (1930) has worked with a fairly polyphagous race which, in addition to causing disease in border Phloxes, will attack eight other species of plants belonging to five different genera.

Amos (1919), Goodey (1922), Ware (1925) and Hodson (1926) have published papers dealing with the occurrence of biologic races of this nematode in England. Amos, in twelve attempts, found that he could not set up disease in oats with a race highly adapted to red clover. He also brought out the various susceptibilities of different kinds of clovers and other legumes to this clover race. Goodey carried this line of work somewhat further in 1920 when, working with the same highly specialised race from red clover, he obtained experimental results of infections which enabled him to express in figures an index of susceptibility of various clovers and legumes to this particular race. It may be noted in passing that in these experiments lucerne was not attacked, whereas in U.S.A. and South Africa there is a strain which produces a serious disease in this host.

Ware worked with a race of *T. dipsaci* highly adapted to white clover and found that red clovers were practically not susceptible to attack from it. Hodson experimented with three or four different races and tested their infectivity to different hosts all known to be capable of functioning as hosts to the parasite. From these he found that two strains living on narcissus, one for three and the other for four years, would not attack oats or red clover. An onion strain, known as attacking onions for one year, would not affect oats or clover. A potato race, previous history unknown, attacked potato but would not produce disease in oats. A race adapted to oats and cocksfoot was found to attack these easily but would not set up disease in narcissus or clover. Finally, marked evidence of distinct biologic races is provided by the experiments of van Slogteren (1920) in Holland who worked with the parasite from hyacinths and narcissus and found by observations in the field, by careful experiments in concrete cylinders and by injection experiments that the hyacinth race would not produce disease in narcissus nor the narcissus race set up disease in hyacinths. He was able to set up typical symptoms of disease in narcissus with worms from *Amaryllis formosissima* and *vice versa* from *Ismene calathina* and *Galanthus nivalis*. Hyacinths, however, were not attacked by worms from *A. formosissima* nor *vice versa*; neither was *Galanthus nivalis* attacked by the hyacinth worm.

It would seem from these results that the hyacinth race is more highly specialised to its host than the narcissus. Miss de Bruyn Ouboter (1930) has recently published some results of a very careful study of these two races and claims to have been able to identify and separate them by certain differences in measurements, when treated statistically, and by a very minute morphological difference in the appearance of the lateral lip regions of the head when viewed end on.

(3) *Heterodera schachtii*. This nematode attacks the roots of plants and for many years has been known as a serious pest of the sugar beet in Germany, France and U.S.A. It also attacks several other kinds of cultivated plants including oats, wheat, peas and potatoes.

An interesting experiment was carried out practically forty years ago by Liebscher at Göttingen. A plot of ground was continuously planted with peas for thirteen years, till finally the crop became so badly attacked that it only yielded 4 per cent. of the seed sown. Separated from this by a path about 1 metre wide, was another plot on which oats were grown for seventeen years in succession, until at the end the attack was of a high order. Liebscher then planted both plots with a number of different crops in order to find out their response to the attacks of *H. schachtii*. The result was that on the pea plot all kinds of peas and beans were highly infested, a slight infestation occurred on lupins and *Soya hispida* and no attack on oats, wheat, barley, beet, rape, turnip and cabbage varieties. On the oat plot none of the peas, beans or other legumes were attacked, but a heavy infestation was present on oats, wheat, barley, beet, rape, turnip and cabbage varieties. It cannot be considered from these results that either pea or oat strain is remarkably highly specialised to its particular host, since each is capable of parasitising quite a number of other plants in addition to the pea and the oat.

A case is reported from France by Marchal and Capus (mentioned by Steiner, 1925) in which *H. schachtii* attained such a high degree of specialisation on peas that it would scarcely attack sugar beet.

There is in Germany, Sweden and this country a well-marked race of the parasite

so highly adapted to life on the roots of potatoes that it will only attack one or two other kinds of plants with great difficulty. Attempts have been made in this country to get it to go over on to sugar beet, mustard and several other kinds of plants including many species of Solanaceae, but so far without success. A point of interest with regard to this potato race is that the shape of the adult female is almost spherical, apart from the neck region, whereas those forms attacking sugar beet, oats, etc., are lemon-shaped. This difference in shape led Wollenweber in 1923 to erect a new species, *H. rostochiensis*, for the potato form. Triffitt (1928), however, has compared the potato, sugar beet and oat races and finds that the adult males show no morphological differences whatever. Zimmermann (1927) and Goffart (1928) working in Germany have succeeded in getting an infection set up on sugar beet from the potato race. The former planted beet on land infested with cysts of the potato race but got no attack the first season; the next year there was a good infection, and the third year a still heavier one. Goffart (1928) found that the females produced on sugar beet had the lemon-shaped body although originally spherical on the potatoes, and we may conclude from this that the spherical shape of the body found in the potato race is in the nature of a response on the part of the parasite to a particular host.

So much by way of a very brief sketch of a limited amount of the evidence as to the occurrence of biologic races in nematodes. What interpretation are we to give to the observed facts?

The view most generally held up to now with regard to biologic races in nematodes, is that they represent adaptations, such that, the longer a parasite attacks a particular host the higher the degree of specialisation to it becomes and the less is it able to attack other plants.

Steiner (1925) indeed holds that only a population which has lived for many generations exclusively on a given host should be termed a biologic race. According to him, the previous host history of a so-called race or strain is all important in explaining its present behaviour, *i.e.* whether it will attack one or more plants depending on whether it had previously lived on one or several hosts. His view, as I understand it, implies a physiological accommodation of the parasite throughout its successive generations to the host, accompanied, possibly, by some physiological specialisation of its organs of perception whereby it can locate its true host but remains unresponsive to an abnormal host.

The view that adaptation only is concerned has been criticised recently by de Bruyn Ouboter on the grounds that it involves the transmission of acquired characters. She says: "It is thus seen that biologic races are not considered to be genetically different but are adaptations to definite hosts which gradually become more marked during various generations." She adds that "inheritance of acquired characters is therefore assumed here, a hypothesis which has always appeared false." I am not concerned here to defend the adaptation hypothesis, but I do not think that inheritance of acquired characters is necessarily involved in this kind of adaptation to a host. It does not seem that by continued life on one kind of host we have a state of affairs exactly parallel to and on all fours with the inheritance of acquired characters. I incline more to a view that this specialisation to a particular host can be better explained by what one may term, for the want of a better expression, a food-memory hypothesis. This implies the passing on to the offspring of what we might call a factor X, characteristic of the particular host and predisposing the larval worms to seek that host. One may

even go further and surmise that if, as I have already suggested, a physiological specialisation of the sense organs occurs whereby the worm locates its true host, then this memory factor X would be linked up to the chemotactic stimulus correlated with the sense organ for detecting it. Such a memory factor might quite well be located in the somatic tissues of the offspring, and the hypothesis does not involve or imply any essential modification or alteration of the germplasm. Inheritance of acquired characters, on the other hand, does involve genetical modification.

In place of the view that biologic races are to be accounted for by adaptation, de Bruyn Ouboter advances the suggestion that the observed facts are better explained on the assumption that such races have arisen by selection from a mixture of genotypically different material and by mutation. An example or two will suffice to illustrate her hypothesis.

Ritzema Bos found that buckwheat, after being sown in soil infested with *Tylenchus dipsaci* of a rye strain, was not diseased the first year; in the second year there was some disease and in the third plenty. In this result he saw an adaptation of the rye race to buckwheat. The facts, according to de Bruyn Ouboter, can just as well be explained by selection. Assuming that she began with a mixture of homozygotes which can live only on rye and heterozygotes with a capacity for life on both rye and buckwheat. The latter will occur only to a slight extent because the homozygotes for buckwheat always die off so long as they have only rye at their disposal. When buckwheat appears, however, it is just these homozygotes which continue to live as soon as they arise by bastard splitting. At first they are few in number but speedily increase on their appropriate host. With regard to my own results which exhibited different degrees of attack on different clovers, etc., she suggests that the results can be explained on the assumption of the presence of a population containing many individuals with a gene for red clover, fewer with a gene for alsike, still fewer with a gene for white clovers and no individuals with a gene for lucerne and trefoil.

In Hodson's experiments the fact that races would not pass over to certain other plants is to be explained, according to her, on the ground that by continuous life on one host, inbreeding had brought about races of homozygotes.

Van Slogteren's results are accounted for on the assumption that he worked with eelworms from hyacinths without genes for narcissus and *vice versa*. Experiments showed, however, that the narcissus worms possessed genes for three other plants at least. With regard to the origin of the narcissus race, it is suggested that it might have arisen by mutation from a race capable of attacking both hyacinth and narcissus.

I am not committed to either adaptation involving a food-memory hypothesis, or the selection of genotypes as the method of origin of biologic races but put them forward for what they are worth; there is a good deal to be said for each, but time is too short to enter upon the merits or demerits of either.

Before finishing, however, I should like to raise one other question, namely whether we are to recognise the existence of biologic races in nomenclature and if so how? If we look upon biologic races as those in which constant physiological differences exist can we give expression to this in our names of organisms? The use of a third name, *i.e.* that of the host, has been adopted by some. Thus in the case of the polyphagous race of *Tylenchus dipsaci* capable of parasitising fifty-two different kinds of plants we should simply use the binomial system, whereas for the race highly specialised to the hyacinth we should call it *T. dipsaci hyacinthi*.

De Bruyn Ouboter, in fact, takes the view that the biologic races of *T. dipsaci* on hyacinth and narcissus are comparable, to a certain extent, to ecological races of free-living animals or plants, and in this sense uses the host name in addition to the ordinary specific name. One must, I suppose, be content to name an organism by the accepted binomial system *pro tem.*, until one has had time to carry out an elaborate piece of research to determine whether it belongs to a biologic race and when this is done, affix the host name to it.

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III. BIOLOGICAL RACES IN FUNGI AND THEIR SIGNIFICANCE IN EVOLUTION[1].

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Sub-specific groupings have been known in the fungi for over thirty years and, during this period, a great part of mycological and pathological research has been concerned with questions of differentiation and classification, modes of origin and genetic relationships, morphology and physiology and parasitism in relation to the species and condition of the host plant in different environments. The problem of biological races is, in fact, one of the largest and most widely reaching problems in our science and is of fundamental importance to theory and to practice.

In many groups of plants and animals the existence of biological races is still a moot point, but their occurrence in the fungi is now so generally recognised that one tends to assume it in species even where it has not yet been demonstrated. One almost presumes that in such cases an intensive study would reveal their presence, and that they are still unknown for the simple reason that they have not been looked for.

The classical example of biological races in the fungi is the black rust of wheat, *Puccinia graminis*. This species is subdivided into a number of varieties which, in certain cases, show slight morphological differences but which are separable primarily by their parasitic relation with host plants. Each variety can attack several species of one or more genera of grasses, but it cannot invade certain species of other genera, which are attacked by other varieties of the fungus. Thus *P. graminis tritici* is parasitic on wheat, barley, rye, etc., *P. graminis secalis* on barley and rye, etc., but not wheat, *P. graminis avenae* on oats, etc., but not wheat, barley or rye, and so on. Further, each variety may comprise several physiological forms which differ in their parasitic qualities. Thus *P. graminis tritici* contains more than fifty such forms which may be differentiated by their effect on certain varieties of *Triticum* spp.; *P. graminis secalis* has about a dozen, whilst in other varieties such as *P. graminis phleipratensis* no physiological forms have, so far as I know, been demonstrated. The situation is, however, even more complicated than this, since cultures of the same physiological form obtained from different sources may be quite different in genetic constitution.

What is true of *Puccinia graminis* is also true in varying degree and with various modifications in systematic values of numerous other fungi. In certain cases the immediate intraspecific divisions are separable on morphological criteria and may be classed as varieties. In other cases more or less distinctive morphological characters may or may not exist, but they are not feasible criteria and specific subdivision depends on parasitic qualities or, where a fungus can be grown *in vitro*, on cultural

[1] The problems of racial specialisation in fungi were so adequately treated by Stakman in his chapter of the volume *Plant Pathology and Physiology in Relation to Man* (W. B. Saunders Co., London and Philadelphia, 1928) that there seemed no virtue in repetition. Stakman, however, concerned himself principally with the empirical data and I have therefore written this paper with a theoretical bias—it may perhaps be regarded as a theoretical appendix to his. Stakman's essay contains an excellent bibliography. The more recent paper by Stakman *et al.* "Mutation and Hybridization in *Ustilago Zeae*" (*Univ. Minn. Agr. Expt. Sta. Tech. Bull.*, 65, 1929) and Schaffnit's paper "Über das Spezialisierungsproblem bei parasitischen Pilzen" (*Angewandte Bot.* 1928, x, 2) should also be consulted.

characters and behaviour. Using purely physiological or biochemical criteria these smaller groups may be further divided until, with increasing accuracy and fineness of criteria, one would, I suppose, arrive at individual lines. The difficulty, and it is a fundamental one in this pursuit of precision, is that one becomes increasingly uncertain of one's values and even of one's empirical data.

There is much discrepancy in the nomenclature of these smaller groupings and this is becoming a source of confusion. The procedure I adopt in my *Botrytis* work is as follows. Each separate pure culture made by direct isolation from fresh material, whether a number of cultures are made from a single lesion or from one or more host plants, I term an *Isolate*. If the first culture direct from the diseased tissue contains, as is very often the case, two or more types, the pure or single-spore isolations from this mixture and not the first impure culture are the isolates. Each isolate is an individual line and sub-cultures are merely duplicates or replicates of that isolate or line. The isolate is the nearest equivalent to Lotsy's "species" and in spite of Ramsbottom's playful remarks of some few years ago, there is theoretically much to be said for Lotsy's position.

When the isolates are compared on various culture media and under different environmental conditions they fall into a number of groups each of which contains isolates which are alike in their cultural characters and cultural behaviour. Each of these groups constitutes a *Strain* or a *Race*, and equates with Lotsy's "Jordanon." In *Botrytis cinerea* which is an asexual fungus[1] the races are analogous to Turessen's "apomict biotypes," whereas, in a heterothallic fungus such as *Mucor*, the races correspond with his "amphimict biotypes." Within a race there may be and very often are isolates which, although indistinguishable on cultural criteria, are yet different in their parasitic qualities, and these I call the *physiological forms*. Even two apparently identical physiological forms may still show differences in their biochemical attributes. The physiological form in *B. cinerea*, like that in *Puccinia graminis*, corresponds with Turessen's "ecotype." When the races or strains of *Botrytis cinerea* are compared they fall into somewhat elastic cultural groups which I call *varieties*, and the total of these varieties is the Species, which corresponds with Lotsy's "Linneon." *B. cinerea* is also equivalent to Turessen's "agamospecies," whereas such a fungus as *Neurospora sitophila* corresponds with his "coenospecies."

If one compares the above terminology with that suggested by Wollenweber *et al.* for the Fusaria, the species *B. cinerea* is analogous with the *Fusarium* species. My varieties in *B. cinerea* are elastic cultural groupings and are not quite equivalent to the morphological varieties in *Fusarium*. In *B. cinerea* I can, as yet, find no good evidence of the existence of morphological varieties. The *forma* in *Fusarium* correspond in general with the races or strains in *B. cinerea*.

The individual unit or line is the isolate and if, adopting one or other particular character as a criterion one arranges a large number of isolates by degree of resemblance, they fall into a particular order. A different criterion usually gives a different arrangement of the same isolates. One cannot say, therefore, that isolates *A* and *B* are more closely related to each other than *A* and *M* because *A* and *B* are alike on one medium and *A* and *M* are different on that medium. On a second medium or under different conditions *A* and *M* may be more alike than *A* and *B*

[1] Many years' study of *B. cinerea* has given me no evidence of a connection with Sclerotinia.

since different isolates are not influenced in the same way by the same medium or conditions. My experience has led me to the view that the relation to each other of the races composing the species *B. cinerea* and the relation of the several species composing the genus *Botrytis* are impossible of representation in the conventional phylogenetic-tree diagram. There are no branching systems showing linear progression but irregular clusters in time, and the fact that the modification orbits of two strains overlap more than those of two others does not imply any closer phylogenetic relationship.

The value to be attached to both specific and sub-specific groupings is a difficult question for it immediately raises the fundamental issue of the "species concept." If one looks generally over the systematic treatment of the fungi it becomes clear that there is no single "species concept," for the described fungal species include natural and unnatural units of widely different kinds. I am not thinking here of the obvious difference between unnatural but convenient taxonomic species and natural species but only of the latter category. Unfortunately, in systematic mycology the two categories have been mingled together in almost inextricable confusion. There is no agreed level of characterisation which defines "the fungal species" and the degree of morphological difference seems to be of little or no importance in distinguishing species and lower units. A particular level of characterisation delimiting species *A* might, in some other genus or even in the same genus, confer upon organism *B* the rank of a variety or, again, might raise organism *C* to generic rank. The genera *Botrytis*, *Fusarium* and *Phytophthora* are good examples of the systematic confusion attendant upon the absence of an accepted specific evaluation. Further, in certain groups such as the rust fungi, physiological forms are often separable with a degree of precision exceeding that of species differentiable on morphological grounds in many other fungi. It is, in fact, simply herbarium convenience and traditional interests that demand a morphological criterion, but, although I can see no philosophic reason why morphology rather than some functional quality should be primary, there can be no doubt that in a pragmatic philosophy of biology morphology is primary and in our narrower field of fungal systematics must be the first criterion.

Let me illustrate the species difficulty more fully by taking an example from my own work, and I may say that *Botrytis cinerea* will be a unifying thread running through my remarks to-day. The species *Botrytis cinerea* may be visualised as, at any one moment, a cluster of numerous races or strains morphologically congruent on the host plant but *in vitro* showing marked and constant cultural differences. The cluster of races which is *B. cinerea* is disparate from the several clusters of races which compose other "natural" collective species in the genus, such as *B. alii*, *B. polyblastis*, *B. tulipae*, etc., which are again disparate from each other. In the collective species which, for my own convenience I call *B. tulipae*, I include a large number of microsclerotial races which occur principally on liliaceous hosts and which in many cases have received specific names such as *B. narcissicola*, *B. galanthina*, *B. gladioli*, *B. hyacinthi*, etc. The collection of numerous additional isolates shows no sign of filling in the spaces between the specific clusters but simply increases the density of one or another specific cluster about what may be, perhaps, visualised as focal points or loci. All the evidence I have obtained suggests that although the clusters may overlap yet they are distinct and separate from each other, *i.e.* that each species is a natural grouping. It will be clear that, in my conception of the *Botrytis* species, the herbarium type

species has practically no place, for it is merely a single phenotype of one of a greater or lesser number of races composing the species.

Duplicate sub-cultures of an isolate usually show slight differences, but in a large series of replicates these differences fall into a normal range of variation. The normal variation of a quantitative character, such as spore length, can be expressed in a frequency curve with its minimal, modal and maximal values and a mean value which characterises the isolate under the particular conditions. The cultural or behaviourist characters are, however, largely qualitative and cannot usually be measured and expressed in a frequency curve or by a mean value. Yet it is plain to the experienced eye that these characters show the same phenomenon of normal frequency variation. What might be called one's "intuitive" estimation of the cultural characters of an isolate is a behaviourist mean value, a cultural statistic rather than a mathematical one. When one has had lengthy and intensive experience of large numbers of isolates of any particular race or species one's eye judgment of cultural mean values becomes extraordinarily exact, quite as exact and finite within certain ranges as physical measurement. In fact one's eye judgment is akin to an aesthetic valuation, a direct intuitional judgment. The particular point I want to make, however, is that in dealing with isolates or races or species we are never dealing with type or individual values but only with mean values which we generalise for systematic purposes. Thus, the species is a morphological average value, the race is a cultural average value, the isolate as characterised by its spore dimensions is a statistical average, whilst the physiological form is a parasitic average. Further, as in all such cases, the mean value may not fit any single individual. We shall also, I think, need to recognise in biology something analogous to the physical principle of indeterminacy.

If one had isolates of only five or six races of *Botrytis cinerea* their differences could easily be so great that one would almost unhesitatingly call them species, as has been done with races of the "liliaceous" cluster of *Botrytis* forms. When, however, one compares hundreds of such and allied races it becomes clear that the strikingly different isolates are only members of widely disparate races in the enormous cluster which is the species *B. cinerea*. Yet I am quite sure that numerous accepted species of fungi have no greater claim to specific rank than have many of the races that I should include in the species *B. cinerea*, or than have those forms which I should degrade to racial status within the natural collective species *B. tulipae*. I think this is true of many species in the Hymenomycetes, in such genera for example as *Armillaria* or *Russula*, and it is quite obviously the case in many Ascomycetes and Phycomycetes and quite absurdly the case in many Fungi Imperfecti where the position is still further complicated by the presence of numbers of purely taxonomic species and genera. In his monograph, Gäumann has given specific rank to the races composing the several collective species of the genus *Peronospora*, and in general the tendency in mycology has been to ascribe specific rank to any form recognisably distinct from other known forms however slight or minute the distinguishing character. In many cases the genetic stability of the character has been tested but, in very many cases, it has not even been questioned especially in species described straight from "nature" or from herbarium specimens. To my mind it is not feasible to adopt this procedure with the genus *Botrytis*, for the promoting of the races of *B. cinerea* alone to specific rank would lead to an impracticable number of new combinations. And yet from a pathological standpoint racial differences within a collective species may

be infinitely more important than morphological differences among different species. It is, sometimes, not very helpful to the pathologist merely to call a fungus *Peronospora parasitica* or *Botrytis cinerea, Erysiphe polygoni* or *Puccinia graminis*: the essential and useful name is that of the race or the physiological form and we must find some acceptable method of distinguishing such forms and races in the literature.

Many cultural races of *B. cinerea* are also biological races since they differ from each other in their parasitic relationships. On the other hand, certain races which are distinct culturally seem, so far as I can discover, to resemble each other in their parasitic qualities. Further, isolates which are separable on parasitic criteria may be congruent in their morphological and cultural characters. That is to say there is no necessary correlation, so far as I can see, between morphology, cultural characters and more purely physiological qualities. Of course in many instances such a correlation may be very evident, but in others its absence may be equally well marked. The situation is, further, vastly complicated by the fact which is usually unsuspected or overlooked, that races very often occur not individually but in mixed populations, two or more races harmonising so intimately as to appear and behave as a pure individual growth. This phenomenon can be reproduced in the laboratory *in vitro* where certain mixtures of races are found to grow harmoniously and others disharmoniously. The relation of such populations and of the balance of their component strains to disease is a question of great importance which, to-day, I must pass by.

Intra-specific categories of certain fungi such as the rusts and powdery mildews are, as yet, only determinable on a basis of host relationship. In a great number of fungi, however, where development *in vitro* is possible, the cultural characters are the simplest and most obvious criteria available for sub-specific classification. Cultural characters are not biological quite in the sense that parasitic or other physiological qualities are, unless one regards a medium as equivalent to a host plant, nor are they morphological in the usually accepted sense: one may regard them as behaviourist criteria. Still, for our purposes cultural races may be equated with biological races in the more strict sense, and this is in fact the practical usage of mycologists.

Now, as I have said, mycologists take for granted the existence of biological races much as I have described them in *Botrytis cinerea*, and the questions which interest us are those of origination, variability and genetic relationships, of cultural and physiological qualities, of parasitic relationships. In fact the questions are just those which we are facing in our discussion to-day. How do races arise? Are they fixed and permanent? If they change why and under what conditions do they change? Do they change in one or in more characters, in one or in more directions? Are there limits to their changeability and if so, what and why? And, finally, what relation do these questions bear to pathological theory and practice and to the problem of fungal evolution?

Generally speaking new forms may arise, theoretically, in several ways; by adaptation of an existing form, by hybridisation of two existing forms or by some other mode of genetic fusion and segregation, and by mutation. Of course the question is not quite so simple as this, since what is apparently a new form may possibly be only the re-emergence and stabilisation of a suppressed or latent character or grouping of characters or of a particular cyclogenic phase in a polyphasic organism. Further, and this is a point of view that I feel to be of the very greatest importance in this field of mycological inquiry, hardly any empirical phenomena of fungal variability

can be accepted at their face value. The more experience I have of this field of study the more I realise the difficulties of extracting and interpreting what are the real facts underlying apparently simple phenomena. In perhaps most fungal researches no attempt has been made to penetrate below the empirical surface. Some of these difficulties are, I think, present in bacterial research, but the more individualised organisation of helminths, insects and the higher animals and plants necessarily eliminates them in study of those organisms. Some of the reasons for this viewpoint will be clear from what I have already said and others will become clear as I proceed. Many of the questions before us were discussed in some detail in a paper read to the International Congress of Plant Sciences at Ithaca, in 1926, so, as my time is limited I may perhaps be allowed to speak rather more generally to-day.

The permanence or changeability of fungi either in pure culture *in vitro* or maintained on host plants has been a subject of intensive enquiry for over three decades, and opinions as to causation and interpretation still vary widely. Most of the earlier work and much of the more recent work does not take into account a fact which is being increasingly recognised and which seems to me to be of primary importance. This fact, which I have already mentioned, is that both *in vitro* and on the host plant two or more races may grow commingled to form so harmonious a population that its complex nature is not suspected. Change in such an apparently pure growth may not be due to any process of adaptation or mutation but to the selection by environmental factors of one or another genetic component of the population. It is the old difficulty of diversity simulating variability.

The more experience I have of the fungi the more common and important I feel to be this process of selective elimination. My experience is compelling me to think that in the field the races of *Botrytis cinerea*, for example, occur quite generally as more or less mixed populations, and that this is also true of most other parasitic fungi. Direct isolations from such diseased plants very often give apparently pure cultures which can be analysed by single-spore methods into two or more races. When working with cultures *in vitro* the only sure method of separating the components of a harmoniously integrating population is single-cell isolation, for single-colony isolation may not differentiate the components even after numerous transfers.

The question of fungal adaptability or educability goes to the very roots of our problem, and most of the controversy has centred in the powdery mildews and the rust fungi. These organisms cannot yet be grown *in vitro* and, as cultural criteria are unavailable, the only practicable basis of racial distinction is one of parasitism and host relationship. If the bridging host theory be correct and the parasitic qualities of a fungus can be altered by adaptive passage, then parasitic qualities are not valid criteria, in which case the sub-specific classification of these and certain other fungi is chimaerical. This is not the place for considering in detail the precise evaluation of this or that datum, but in my own opinion there is no evidence for the bridging host theory which is not better interpreted on a basis of selective elimination. One has only to visualise the older work in terms of recent studies to see the greater adequacy of selective elimination within a harmoniously integrating population under changed conditions as an explanation of empirical phenomena only explicable previously by a bridging host or an adaptational theory.

What I have said of the rust and powdery mildews is, I think, obviously true of numerous other fungi: the fungal genotype is unaltered by education. I want, how-

ever, in this connection to sound one note, which may or may not be of importance depending upon its scope and width of application. I have carried out numerous experiments with *Botrytis cinerea*, growing pedigree isolates (*i.e.* isolates which have been repeatedly single-spored and which show no signs of instability) for long periods under different physical and nutritional conditions both *in vitro* and on living host plants. At the end of the experiments I have brought these differential cultures back to growth in a common standardised environment. Under different conditions the particular isolate may show different phenotypic expressions, but, when the isolate replicates are returned to a common condition, the reversion is usually immediate and complete. Occasionally, however, there is a more or less well-marked lag period which may be transient but which may extend to one, two or even three transfers. In an extensive experiment in which spore size was the criterion and in which, so far as I can see, any personal equation was entirely eliminated the statistical evidence for a lag period was significant. On return to the original conditions, however, reversion to the original mean dimensions occurred in a very few transfers. The evidence in this particular experiment was more suggestive of a physiological unbalancing of the internal or cytoplasmic environment with subsequent re-accommodation than of any influencing of the genotype. The outstanding phenomenon was not the slight lag period but the reversion exactly to original dimensions. This type of change resembles a lingering suggestion of the previous phenotype which persists through the reversionary change to parental facies, something analogous perhaps to the faint traces showing through a palimpsest. In the absence of minute and experienced scrutiny or of a considerable series of exact measurements, it would be, and in my experiments was, overlooked in the dominating reversionary change.

With this qualification, however, all the evidence seems to point to a fixed genotype with phenotypic modifiability and immediate reversion to original type on return to the original conditions. If the causation of the lag period which I have mentioned be genotypic it may, perhaps, be due to something in the nature of a labile oscillation of factors such as Baur has shown to occur in *Antirrhinum*.

The frequently described adaptability of yeast species seems to me, again speaking rather generally, to be better interpreted in terms of selective elimination. Yeasts, *in esse* or *in posse*, are sexual Ascomycetes, and the brilliant work of Dodge on *Neurospora* indicates something of the amazing genetic complexities possible in a sexual Ascomycete and the range of permutation and combination of segregating characters. Selective elimination by the environment acting on a yeast population derived from a single heterozygous cell could, I suggest, give rise to all the empirical phenomena which have been described. At any rate this seems to me a far more probable explanation than that characters have arisen *de novo* by adaptive processes.

The fine work done during the last ten years on the genetic analysis of the Hymenomycetes, rusts and smuts gives evidence of the possibility of an almost infinite genetic complexity with the production of endless new forms arising in the processes of hybridisation and segregation. In neither the Ascomycete nor Basidiomycete work however, is there, so far as I know, any evidence that the new forms arising in hybridisation are of higher status than varieties of the parental forms. If one takes a conservative attitude towards species evaluation, hybridisation does not seem to be the *vera causa* of evolution in the fungi but only a process of varietal diversification. If, on the other hand, one gives specific rank to races, one has species in the making

and evolution is proceeding apace. It all depends on one's point of view as do so many of one's conclusions in biology and to this I shall return later.

One further issue, relevant to our subject, illustrates well some of the difficulties inherent in the study of fungal genetics to which I have referred. It is not easy to think of conditions in the Hymenomycetes, for example, in terms of sexual fusion. What we seem to have is a capacity for selective fusion better considered in terms of copulation or of self-sterility factors. The genetic phenomena, however, are those associated with sexual fusion, save that the possibilities inherent in the resulting combinations and segregations of not two sexual groups but four and even unto twenty-four sexual groupings, as Hanna has shown for *Coprinus*, are almost inconceivable. And to complicate the issues still further there is the diploidisation process recently described by Buller or the nutritive heterothallism hypothesised by Gwynne-Vaughan. There is certainly no lack of material whereon selective elimination may operate.

The possibilities of genetic complication in the fungi seem to me impossible of exaggeration, surely exceeding by far those in any other group of organisms. As I read the evidence, however, I do not see any data which demand a theory of the origination of new forms by genotypic change on an adaptational or Lamarckian basis. All the data which are explicable on such a theory are, so far as I can see, better fitted by terms of selective elimination in a genetically impure population.

Most mycologists would admit the hybrid production of new forms of varietal and lower rank and there is now convincing evidence on this point for all the major groups of fungi. If one did not know of their laboratory origin many of these new combinations would be given specific rank, and it is of considerable interest that by crossing different strains of *Dictyuchus*, Couch has obtained variations which invalidate a number of previously accepted species of that genus. New combinations arising as a result of hybridisation do not seem to me, however, either sufficient to explain the data or to exhaust the obvious possibilities inherent in the fungi.

One of the most commonly studied types of variation in the fungi is that which is still frequently called "mutation." Recently many writers have followed Stevens and used the term "saltation." Personally, I prefer to remain entirely non-committal, and so use the merely descriptive term "discontinuous variation" which has no genetic implications. Variations, which are apparently discontinuous and appear as sectors or secondary colonies in a plate culture, may be produced at will by suitably mixing two or more races of a particular fungus or by treating in various ways a harmoniously integrating population comprising two or more races or by differentially treating an isolate culture. Such mechanical analysis has often unwittingly been involved in this kind of research. The real problem, however, is not this false sectoring but is the spontaneous arising of such sectors or secondary growths as discontinuous heritable variations in an isolate culture where there is no question of differential conditions or of an initial commingling of races.

I may say at once that we are entirely ignorant of the genetical and cytological bases of the phenomenon of discontinuous variation in the fungi. We know nothing whatever about gene mutations, chromosome transformations, aberrations and rearrangements and all the cognate cytological phenomena the investigation of which is throwing such a flood of light on the so-called mutations in higher organisms. We are more urgently in need of information on these problems than on any others in fungal

genetics. It is because of our profound ignorance of these matters that I avoid the term "mutation" and adopt the non-committal words "discontinuous variation." If the discontinuous changes that have so frequently been interpreted as mutations throughout the fungi are really of the nature of qualitative gene changes comparable, perhaps, to the point mutations in *Drosophila*, then the fungi in general are, I think, undoubtedly the most highly mutable of all known organisms. If they are not regarded as qualitative gene changes and the term "mutation" is simply used in the old omnibus sense, then it may cover dissociative, cyclogenic, segregative, combinative, and any other type of causation and it has no analytic significance. Its use, to-day, in this sense is, I suggest, mere obscurantism to cover our ignorance of any feasible mechanism of hereditary change, for I believe that progress lies in the correlating of every detailed occurrence with its antecedents in a perfectly definite manner exemplifying general principles.

I would rather say phenomena than phenomenon of discontinuous variation, for it seems to me plain that the sectors and secondary colonies which turn up so commonly in our pure fungal cultures are the visible expressions of not one but many complex processes which may be quite separate in causation.

Further, I would emphasise that the almost universal assumption in mycology, as also in bacteriology, that a culture of single cell origin is necessarily pure in a genetic sense is a perfectly gratuitous assumption and one which is negatived by a vast and increasing weight of empirical evidence. Genetic purity is presumed and in consequence unexpected deviations or discontinuous variations are regarded as evidence of mutation. Knowing what we do of the infinite possibilities of genetic contamination and the extreme difficulty of ensuring and still more of proving genetic purity surely it is clear that the basic presumption should be one, not of genetic purity but of genetic impurity, and variations should be regarded simply as evidence or proof of that impurity.

In *Ustilago Zeae*, Stakman *et al.* have described the production by a single haploid clone, in a period of twelve months, of 220 mutants, 162 of which were different. If they found 220 it is quite safe to assume that they missed at least an equally large number in which the change might have been in biochemical or other characters not easily recognisable. Personally I see no reason why that very prolific clone should ever cease to be in labour. Leonian has described equally fecund parents in *Fusarium* and *Phytophthora* and such phenomena seem to me to reduce almost any hypothesis to an absurdity. When, however, one considers the smut fungi and realises their immense possibilities in cross-breeding and heterozygosis, I must confess that I still feel hybridisation to be a more probable explanation of the phenomenon than mutation, although of course there is no reason why the latter should not also occur. The difficulty is to prove it, and Stakman and his collaborators have gone further in this direction than most other workers who usually remain content with description of empirical phenomena. Perhaps I should say that my views on mutation and the difficulties inherent in its proof are strongly influenced by those of Lotsy.

Further, in the higher organisms segregation is commonly effected at the reduction division, but evidence is steadily accumulating which suggests that, at least in plants of many kinds, as Bateson insisted years ago, comparable segregations occur in somatic divisions also. Chromosome quantitations, dislocations and transformations giving rise to somatic segregation in fungal hyphae would originate a complex popu-

lation on which selective elimination could operate. Again, as Lotsy has repeatedly pointed out, homozygosis is no evidence of non-hybridity and the haploid nucleus can have and very frequently has a multiple origin. Somatic segregation, therefore, even in a haploid clone, might originate just as many apparently new forms as in a diploid mycelium, a factor which should be considered in researches on the variability of haploid clones in Hymenomycetes, smut fungi and other heterothallic forms.

The origin of discontinuous variants in fungi and particularly, perhaps, in such forms as *Fusarium*, *Botrytis*, *Helminthosporium*, etc., may, I think, have still another explanation in addition to those I have already indicated. In my paper to the Ithaca Conference of 1926 I made this suggestion, which is a very obvious one, and I wish to repeat it, for I think the idea may explain much that is still obscure in relation to discontinuous variation and the arising of apparently new forms in the fungi. I would suggest that the function of sexuality with its genetic consequences is, in many fungi, taken by hyphal fusion of a somatic nature. This would result not in heterozygotic but in heterocaryotic and heteroplasmic states whose degree of complexity would depend upon the nature and number of the contributing elements. The permutations and combinations inherent in this type of fusion, which is one of the commonest phenomena in mycology, would, by reason of the modes of cell division, growth and reproduction peculiar to fungi, provide a basis for most of the phenomena of variation which have been observed. Such fusions would be particularly important in forms with multi-nucleate cells and simple conidial reproduction and it is, as a matter of fact, in just such forms that discontinuous variation seems to be especially prevalent. Further, in many fungi the nuclei and protoplasm of two and, in certain fungi, of three, four or five races or even species may become commingled or associated in a single hypha. Dissociation in the growth of such genetically complex hyphae would be the initiation of a sector. The experimental proof of such a hypothesis by synthesising a "mixochimaera" and then obtaining dissociative forms is naturally a difficult task. It would obviously depend upon a nice apposition of forms under precisely correct conditions, with the subsequent chance which, under laboratory conditions, must be rare of isolating just such cells as on further development might be recognisable as dissociative forms. Still the theory is not incapable of experimental proof although the task is much more difficult than Leonian, who has essayed a few naïve and preliminary experiments, seems to have realised.

In actual fact the degree of discontinuous variability, ranging from forms which seem entirely stable to forms which are ever-sporting, is characteristic of the individual isolate. Neglecting other causes of variation for the moment, the invariable isolates would, on the mixochimaera hypothesis, be homocaryotic or homoplasmic lines, whereas the more or less variable isolates would be heterocaryotic or heteroplasmic lines in which the degree of dissociation might reflect the degree to which the cells were genetically contaminated. Sexual fusion occurring subsequently in such forms would naturally still further complicate the issues. Personally, I feel that until we know vastly more about ordinary hyphal fusion, which is an almost completely unexplored phenomenon of the commonest occurrence, we shall not make much progress in our understanding of the genetic behaviour of the asexual but frequently dissociating forms.

Whether as Leonian suggests, following Hadley's views on bacterial variation, there is any real temporary or permanent stabilisation of particular cyclogenic phases

in a polyphasic organism is an interesting question. Hadley and Leonian use the term dissociation in the above way which seems to me far less apt than to use it as connoting actual dissociation from the associated components in a mixochimaera. In any case the idea of stabilisation of cyclogenic phases is one of considerable interest, as I pointed out in my paper to the Ithaca Congress, and I think it deserves serious consideration by mycologists. It would provide an explanation of many empirical data for which the only other suggested explanations are either an amazing type and rate of mutation, extensive somatic segregation with selective elimination, or dissociation from a mixochimaera. Whichever way one's bias leads one, and whatever explanation one considers the more probable is largely a question of personal bias, I think mycologists must in future pay far more attention to the time factor in fungal cyclogeny. And may I say here that I have little patience with those mycologists who unctuously declare that the time is not yet ripe for speculation, that our only attitude must be one of unimaginative empiricism and our only activity a blind accumulation of data. Of course the time is not ripe for speculation; it never is, for speculations are theories that one does not agree with, whereas it is conveniently forgotten that theories are only speculations that one accepts. The study of fungal genetics has proceeded for forty odd years, and there is an almost overwhelming accumulation of data many of which are now simply being multiplied with the idea, I suppose, that what is said three times must be true. If those with a broad experiential knowledge of these data would speculate a little more and synthesise them in relation to modern biological theory the dull repetitiveness of mycological study might be catalysed into greater fruitfulness. Our science needs more rational thought and fewer childish experiments.

And now let me return to the problem of discontinuous variation with *Botrytis cinerea* as my example. When the phenomenon occurs in isolates a greater or lesser number of variants may arise and these may show different morphological, physiological or behaviourist characters. The variation may be in one character only or in more than one, in which case the variations may be simultaneous or successive. It is to my mind a pregnant fact that in a series of isolates composing a race, certain isolates may show a more or less high degree of variability whilst others may be almost or quite stable. The degree of variability under standard conditions is characteristic of the isolate and not of the race. Of course under different conditions, especially of temperature or nutrition, the degree of variability changes. If one makes large series of replicates of a highly variable and of a constant isolate and sub-cultures these two series in parallel, all the cultures of the one series will tend to show the high variability and the others will be almost or quite stable. Thus degree of variability is not a sporadic quality but an attribute comparable with growth rate, structure or any other recognisable character and itself shows a frequency variation. This reliability of behaviour in replicate cultures of particular isolates seems to be true for many fungi, but does not seem to hold quite so well for *Fusarium* species where replicate cultures, at times, give widely divergent variation rates. More extensive investigation would I think bring *Fusarium* into line. Further, isolates are in my experience constant in their modification orbits about their particular statistical and cultural mean values and vary not in an adaptational way but only in a discontinuous manner.

A clonal culture may repeatedly throw the same variant or the same succession of variants in order, or the variants may arise with apparently complete irregularity

and sometimes in very considerable numbers. These variants may be stable, as in the case of an albino *Botrytis*, which has been in culture since 1917 and has during this period resisted all my attempts to select it for blackness and certain other characters. On the other hand, variants may revert partially or completely and, in plate culture, the reversion may be by change of the entire colony or as is more usually the case by reversionary sectors or secondary growths. Variants may be constant for a larger or smaller number of transfers and then revert partially or completely, gradually or suddenly, or they may again give rise to one or more further variants which in turn may revert. Many apparently stable new forms revert if carried on for a sufficiently large number of transfers and constancy of the order shown by my albino line is, I think, a somewhat rare thing. One *Botrytis* variant for example was constant for 127 transfers extending over nearly four years and then gradually, over about seven transfers reverted to its original characters. Many variants have been constant for a smaller number of generations with subsequent reversion in one way or another. Variants which have changed in several characters may show reversion in all those characters or only in some of them. The frequency with which the variant forms of certain fungi show reversionary tendencies has persuaded Caldis and Coons, Goldschmidt and other workers to regard this type of variation as of the nature of the "Dauermodificationen" described by Jollos in the Protozoa. I would rather place the lag period phenomenon I have described in this category.

In cases where an isolate has given rise to a variant which has remained constant for a time and then shown reversion to the parent, one more or less understands the empirical happenings because one knows their history. I often wonder, however, in how many cases the variation has occurred on the host before one makes the isolation. In such cases one would begin not with a parental line which produces a variant but with the variant itself. The discontinuous variation giving rise to an apparently new form would thus, in reality, be the reversion of a variant to an unknown parental form. In actual practice one always presumes the first condition but the second must surely obtain at least equally often.

The variants themselves may be in a positive though perhaps more usually in a negative direction and it may be that the former are really minus variants reverting to the parental type. It is common for more sparsely reproducing variants to appear in *Botrytis* races, less common for the variants to be of a more densely conidial type. Variants are often less virulent than the original form, although occasionally the variation may show increased virulence and the same holds for other morphological, physiological and cultural characters.

One kind of discontinuous variation which is very impressive in those forms in which it occurs is the constant dissociation, transfer after transfer, of the same type or types of variant. I have, for example, one isolate of *Botrytis alii* which constantly gives rise to the same transient variant. Roberts has described an *Alternaria* which every transfer for 57 generations broke up into the same two forms, neither like the parent which, during this period, was single-spored fifteen times. Then again, there are such phenomena as described by Leonian where "organisms (which) are oscillating between different forms. At one time they appear as one species, and at another time as a different one. The surprising part of it all is that two seemingly different species merge into each other, sometimes one species coming to the fore and sometimes the other; or both may appear at the same time and in the same culture." The constant

recurrence of one, two or more perfectly definite variants is known in *Phoma*, *Diaporthe* and many other fungi and is extremely suggestive of segregation or dissociation from a genetically complex parent.

The question of degree or extent of variation is very difficult. Every character, of whatever kind, that one can recognise in fungi seems to be variable and, as I have said, the change may be in only one inconspicuous feature or in numerous characteristics, so that the new form diverges widely from the parental type. Is there, however, in the accumulation of data on fungal variability any evidence that a single variation or succession of variations has produced a new form of generic or specific rank or are all the new forms of varietal or racial value? The direct question is fallacious since one student's race is another student's species and a variant which might be classed as of racial value in one fungus might be regarded as of specific or even generic rank in another: especially if the determination be made by a systematist ignorant of its laboratory origin.

In the variations that I have observed in *Botrytis* races no one has given rise to a new form of specific rank. This, however, is because I have had in culture a great number of races and so have a wide basis for comparison. Had I had only a few races for comparison my point of view would have been very different and I should probably have described a number of new species. All my *B. cinerea* variants fall within the *B. cinerea* orbit; all my *B. alii* variants within the *B. alii* orbit and so on. In numerous cases the variants have been strikingly different in one, two or often several characters from the parental isolate and if stable would need to be ranked as distinct racial lines. There can be no doubt, I think, that discontinuous variation accounts for the welter of racial lines which are the species *Botrytis cinerea*. I have not, however, in many years intensive study observed any change of so great an extent as would lead me to place the new form either in a new species or to transfer it to one or other of the clusters of races represented by *B. alii*, *B. polyblastis*, *B. tulipae*, etc. The variant types of *B. cinerea* all fall within the *B. cinerea* orbit. If, however, the races were promoted to specific rank and the species *B. cinerea* was discarded or became a genus, then discontinuous variation and the origin of species would be one and the same thing. In the "liliaceous" cluster which I call *B. tulipae* some of the races have been given specific rank and if this be their natural value then certain of my discontinuous variants in cultures of these forms exceed the specific limits. In my opinion, however, these "species" are only equivalent to the races of *B. cinerea* and the natural species is the entire cluster of races which for convenience I call *B. tulipae*. In this evaluation the discontinuous variants fall within the *B. tulipae* orbit. That is they are not species in the making but races in the making and discontinuous variation is not a process of specific evolution but only of racial diversification. It is conceivable that geographic or biological isolation might, in time, cause particular races to assume specific value; but I am aware of no evidence for this in the fungi nor do I think it probable.

How far the conditions that I have described in *B. cinerea* or that may be found in any one species are true generally of other fungi is very difficult to say. In the genus *Fusarium*, for example, it is not easy as yet to define or even to visualise specific orbits, due, possibly, to the overlapping of the several species, but my experience inclines me to think that if one takes a broad view of the species the variants arising in the familiar process of sectoring do not originate new species but only racial types

which fall within a collective specific orbit. Again, it must be quite clear that the evolutionary value one gives to the new forms arising by discontinuous variation depends upon one's systematic views. If one follows Gäumann, Lotsy and numerous other students who allow specific rank to races, then, in the processes of discontinuous variation and hybrid combination one has species in the making and evolution is proceeding at an almost alarming pace. If, on the other hand, discontinuous variation and hybrid combination are simply processes of racial diversification, then we have practically no experimental evidence for fungal evolution and we can only reason deductively concerning it and reconstruct by inferential evidence.

In the genera *Penicillium* and *Aspergillus*, for example, variants have arisen to which new specific names have been given. True, these arose as a consequence of shock tactics, after application to the parental fungi of chemicals, etc. In my own experience heat or chemical shock simply speeds up processes which, under more normal conditions, occur slowly. Still, in the above cases the new forms have been regarded as sufficiently unlike any other known forms as to merit specific rank, but they must, I think, frankly be regarded as merely taxonomic species. Stevens also found that certain of his *Helminthosporium* saltants "differed so markedly from their parent as to far exceed the usually accepted specific limits" and this is obviously the case with variants of *Fusarium, Phytophthora* and numerous other genera. There is convincing evidence that clonal cultures of many fungi can give rise to variants which, in the absence of knowledge of their origin, would certainly be classed as distinct species. When, however, one sees such forms arise, in some cases repeatedly, in cultures of asexual fungi derived from single cells, and sees them, in many cases, revert again to the parental type such a view becomes untenable. Rather must one regard these forms purely as taxonomic species which is very unsatisfactory, or as varieties or races in a natural collective species of wide and comprehensive orbit, or merely as dissociative or cyclogenic phases in a genetically impure or polyphasic type.

Recently Wiltshire has described a *Stemphylium* saltant of an *Alternaria*, which is a discontinuous variation from one accepted genus to another. This is, so far as I know, the only recorded instance of so wide a variance, and it may easily prove that the distinction between these genera is not a natural one but only a taxonomic convenience.

All the phenomena that we perceive occur in the isolate which is the procession in time of genetically bound "individuals": the higher systematic categories are deductive abstractions. Practically all the evidence available is of variation within what I consider to be the specific orbit. The few cases of extra-specific variation which have been described are all in fungi in which specific orbits are largely matters of doubt, and in these cases I should simply take a more elastic view of the parental species. Wiltshire's *Stemphylium* variant of an *Alternaria* is a more difficult case, but it seems to me very probable that intensive study might show the genetic or cyclogenic relation of these two forms to be much closer than of generic or even specific value.

It will I think be clear that any views one may hold on the relation of biological races to evolution depend partly upon one's interpretation of variation phenomena in the race and partly upon one's conception of the species. If one considers variation to result from qualitative gene change and one gives specific rank to the recognisably distinct groupings which, in general, reproduce true to type, then I do not see how one can avoid believing that the fungi are in process of violent evolutionary change.

If on the other hand one considers, as I do, that most of the variation phenomena are merely selective eliminations or combinative, dissociative, segregative or cyclogenic changes originating in one form or another of sexual or somatic fusion and one holds that the species in nature is of collective kind, then the facts before us are not evidence of the origin of species and evolutionary progression, but of intra-specific diversity.

The only real event we know is the actual organism, the isolate. Similar isolates compose the strain or race and the range of phenotypic modifiability of the isolates gives the racial orbits. The races cluster about loci in space-time and, as I conceive the process, each cluster is a species, a natural grouping. The specific orbit is, therefore, delimited by the totality of the racial orbits. In turn the genus is given by the totality of the specific orbits and so on for even the higher categories. This view is the outcome of my experience with the fungi and more particularly the species *Botrytis cinerea*, its races and its physiological forms and I think that such an orbital conception of systematic categories and evolutionary relationships, which is a space-time system, is applicable to the fungi in general and perhaps to most other organisms.

IV. BIOLOGICAL RACES IN BACTERIA AND THEIR SIGNIFICANCE IN EVOLUTION.

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On general grounds it might well be believed that, among living things, the bacteria should furnish the ideal material for study of the primary causes of biological change and of the modifying action of the environment on the organism and its heirs.

Here, as almost nowhere else, the germinal and vegetative substances of the race—if they be at all differentiated—are continuously merged in such a way that the former must be directly attained by many of the stimuli and environmental changes which affect the latter. If it be possible to induce change in the idioplasm, here surely is the opportunity to shake it to its foundations. Further, in dealing with bacteria, one may in a ridiculously short space of time study a sequence of generations so vast that the story of Brown-Séquard's guinea pigs and the history of circumcision—the classic evidence in discussion of the inheritance or non-inheritance of repeated mutilations—seem trivial in comparison. If evolutionary divergence depends on cumulative processes, where could these be better studied? Though the possibilities of observation are somewhat limited on the morphological side, serology, pathogenicity and the multifold biochemical reactions of bacteria offer a wide field for the study of adaptation and change.

Yet it must be admitted that up to the present bacteriology has failed to make any signal contribution to knowledge of the evolutionary process. The bacteriologist has been occupied by pressing medical and economic problems; matters of pure biology have had perforce to take second place. Observations on such points as bacterial variation have for the most part been superficial and incoherent; they have noted a material for study without greatly advancing knowledge of the phenomena.

Setting aside technical difficulties, which need not be detailed, the outlook is at the outset somewhat fogged by uncertainty as to what modifications in the substance and behaviour of bacteria are to be regarded as belonging to the normal life-cycle or

cyclogeny of the organism and what are to be accepted as departures from the normal. Bacteriologists I think suffer more from this uncertainty than do workers in other groups. Hadley has recently argued that all or practically all bacterial changes as yet observed are cyclogenic in nature. His monumental work on this theme represents a reaction against the incoherent description of microbic variants, and, as in most cases, the pendulum of reaction probably swings somewhat too far. It is probably best to leave the question for the time being in abeyance and to console ourselves with the possibility that in the bacterial world cyclogeny and phylogeny are not altogether distinct.

The most important line of recent advance in the study of bacterial variation has been in the field of serology. Within the last decade a great deal has been done to explain the discrepant reactions of bacteria to their specific antisera. It has been found that the "typical" antigenic complex of the species may vary in three distinct ways, yielding races in which one or more of the peculiarities are for a time or even permanently established.

In the first place all motile flagellate forms appear liable to lose their flagella and the special antigens associated with these. Aflagellate races of motile species arise spontaneously in nature and in the laboratory and the same condition may be induced by culture on special media. Some of these races—especially those developing spontaneously—are remarkably constant. The occurrence of such variants affords a ready explanation of the existence of non-motile species in characteristically motile groups of bacteria—for example of *B. pullorum* and *B. sanguinarium* in the Salmonella group.

The Salmonella group, which includes the typhoid, paratyphoid and food-poisoning bacilli, presents a second form of serological change. In certain members of this group the flagella occur in two alternative and transmutable states or "phases" which are recognisable by serological means and are intensely distinct. With certain exceptions one phase (specific) is sharply characteristic of the particular type or species which bears it while the other (non-specific) is in large part common to all species which show the phase phenomenon. Each unit of the culture—and as a rule its immediate issue—belongs to one phase or to the other. But between its two phases the organism lives a sort of Jekyll and Hyde existence. Sooner or later, in the vast majority of cases, certain units of the pure phase race adopt the alternative phase and reintroduce mixture in the culture. Occasionally strains are encountered which have a predilection for one phase and maintain their purity through many sub-cultures. In some such cases change may be induced by subjecting the culture to the action of the homologous antiserum, but there are also races derived from organisms of fully developed diphasic character which can neither be lured into reversion by favourable conditions nor goaded into it by specific antibodies. Such instances of fixation of the organism in one or other of its original phases give a valuable clue to the evolutionary history of the Salmonella group.

Besides the diphasic forms, and the aflagellate types referred to above, there exist a few species which frankly correspond in their serology with the non-specific phase of diphasic forms and a larger series which equally frankly correspond with the specific phases of *particular* diphasic species. Careful study of the evidences of relationship between the members of the group leads to the conclusion that the "monophasic" species of Salmonella must have been derived from diphasic forms

by polyphyletic suppression, now of one, now of the other of the original phases—an event which has, as already stated, come under laboratory observation.

The first type of serological variation referred to lies between the flagellate and aflagellate states; the second is a variation of the flagella; the third, now to be noted, concerns the bacterial body.

From the fact that the first observation dealt with variant cultures, contrasting by the granulation and irregularity of their colonies, with the normal parent form, this third type of serological change is commonly referred to as "rough" variation. The manifestations of this change in the various bacterial groups vary considerably, but in those groups in which the question has been sufficiently studied certain common features have been discovered.

The pneumococcus furnishes the most fully investigated case. The I, II and III types of the pneumococcus differ sharply in their serological properties: each injected into animals incites its own specific antibodies. But all these types during laboratory culture are liable to lose their specificity and to become indistinguishable; to develop new serological properties which are common to the whole series. In other words, these three distinct types have a common rough variant. Combined chemical and serological analysis has shown that each normal pneumococcus type owes its specificity to a carbohydrate substance, which in each is in some manner linked to protein—protein which is common to the three types. When the organism becomes rough the specific carbohydrate disappears leaving the common protein basis—linked perhaps with certain receptors common to the types.

The same type of relationship has been demonstrated for the types of Friedlander's bacillus and for the numerous types of the Salmonella group. In the Salmonella group, which boasts some thirty known types the diverse bodies of the normal races seem to yield in roughening what is, from the serological point of view, a single organism.

Rough variants are found in nature and appear during laboratory culture. Among special means of inducing rough change are rapid sub-culture, use of media rich in peptone, the action of immune serum, and exposure to the bacteriophage. The constancy of these forms is always considerable; there is little difficulty in maintaining their purity. Some, however, revert during rapid sub-culture, some during animal passage and many under the action of a suitable bacteriophage. On the other hand some races, particularly races which have been rough for many years, appear to be permanent in their peculiarity.

Rough change, as we can at present see it, involves loss of a specific substance; in pathogenic species it is associated with more or less complete loss of virulence. There is some reason therefore to regard it as a degenerative and retrograde step—almost pathological. Were it not for an isolated item of information roughening might well be dismissed from consideration as a factor in microbic evolution. This single item is in the nature of a miracle. Injected into the mouse the rough pneumococcus is harmless and is speedily destroyed. It has, however, been found that if the injection is made in mixture with killed pneumococci from a normal mouse-virulent culture, the rough form recovers its lost virulence, kills the animal and emerges from its parasitic onslaught as a normal pneumococcus. But this is the marvel: the serological specificity presented by the regenerate organism is said to be, not of necessity that of its parental type, but that of the dead culture with which it was temporarily associated

in the mouse. The serological properties of the III type may be conferred on the rough variant of a type I culture; even rough pneumococci of the heterogeneous and often less virulent Group IV may be, by this means, exalted to pathogenicity.

The form of the experiment—particularly certain stipulations as to the preparation of the killed culture—naturally raised a suspicion that the normal pneumococci employed were not so dead as they were believed to be; but the claim has survived critical study undamaged. It is useless at the present stage to discuss the mechanism of the observed change, but the possibility has henceforth to be considered that roughening may return the stereotyped organism to the evolutionary melting-pot; that from the unformed state of roughness a new specificity may be developed.

Such in summary are the most important facts which have been determined with regard to serological variation. The intensive serological studies of the past few years have given an indication of the way in which closely related species or types differ from one another in substance and of the nature of the bacterial genus. In a group, such as the genus *Salmonella*, each species presents an array of antigenic factors—units of substance—some of which can be separated by chemical means. A number of these factors can be traced through two or more species and in some species the antigenic complex seems to be merely a combination of factors occurring singly in others: the impression is conveyed that evolution has made use of different combinations of certain available materials. Further, the sharply distinct types have an underlying community of substance; a common basis upon which individuality is imposed by the attachment of "side chains." Vaguely, but suggestively, we glean a hint as to the relation of the species to the genus.

Looking back over the ground it must be admitted that with the exception of the transformation of the pneumococcus this serological survey has noted only variation by loss and reversion to the original state. It is true that the literature offers numerous instances of alleged positive change and of transformation of one serological type to another. Some of these claims are so grotesque that nothing but multiplied confirmatory observations could allay suspicion that gross contamination had occurred. In other cases the "positive mutants" can now be readily recognised as loss variants of the types already discussed; in the remainder such forms of variation were not excluded. Up to the present no indubitable instance of addition of a qualitatively new serological factor has been observed.

So much for serology. What of the other traits of the micro-organism? In taking up the question of variation in biochemical activities I go directly to the fermentation reactions: other lines of study have revealed in the main loss variations, and certainly no more striking evidence of positive modification than is demonstrable in the attack on sugars, alcohols and acids.

At the first glance the field of fermentations seems a hopeful one, for under the circumstances of laboratory examination variations of gain have on the whole been more frequently observed than those of loss. The vigorous and varied fermentation reactions of the intestinal bacilli—the coliform, dysentery and Salmonella series of types—have made these organisms a very favourite material for the attempted study of nutritive adaptation.

From many of these organisms races have been isolated which readily attack a sugar, an alcohol or an acid which the parent strain refused to ferment or fermented tardily. Sometimes this change has been brought about by successive transfers

through broth containing the test substance; more frequently the transfers have been made on a solid medium coloured by an indicator to allow selection of the most actively fermenting colonies.

The following series is instructive.

B. coli mutabile grows on lactose agar in large non-fermenting primary colonies which after a time develop within their substance small secondary colonies of actively fermenting bacilli. Sub-culture from the matrix of the primary colony and the phenomenon of primary and secondary growth is repeated; sub-culture from the secondary papillae and you obtain a race which ferments lactose forthwith and in perpetuity. The typhoid bacillus does not readily ferment dulcite or rhamnose, but by culture on the appropriate media tiny secondary colonies of fermenting bacilli may be discovered and isolated in pure sub-culture. But the races so obtained, though they split dulcite or rhamnose, as the case may be, quite rapidly, are very apt to lose their new property when removed from the special medium. Like other members of the Salmonella group to which it belongs, the typhoid bacillus makes no attack on lactose. The feature is so constant that we depend on it in selecting these organisms from first cultures of pathological material. Yet lactose-fermenting races of *B. typhosus* have been developed during protracted sub-culture on lactose media. Again the phenomenon of primary and secondary growth is discernible but the process of lactose culture and selection has had to be repeated again and again over months or even years before actively lactose-fermenting races have been obtained. Even so, the character so tediously acquired is ephemeral; it fades when lactose is for a time withdrawn, though apparently the reverting races are more readily brought back to activity than are typhoid cultures which have not been through the process.

In the case of *B. coli mutabile* and of *B. typhosus* on dulcite and rhamnose the phenomena might very well be interpreted as due to sudden mutation—an adaptative mutation perhaps—with subsequent selection, either by hand or by the medium, of the variant race. The development of lactose-fermenting ability on the part of the typhoid bacillus has on the other hand all the appearance of a gradual education. Yet the phenomena have so much in common that it is difficult to believe that any fundamentally different type of change is concerned in the two groups.

These and other like departures from the usual behaviour of the organism as encountered in nature are generally accepted as evidence of the plasticity of bacterial species, of the acquirement of new and adaptative properties either in response to environmental stimuli or as a result of selection. In a sense this is probably the case: but are the instances cited—and they are as good as any of their kind—truly progressive changes? The fact is that lactose fermentation is typical of the coliform bacilli and that *B. coli mutabile* is exceptional in its partial failure to attack this sugar; that, among Salmonellas, the typhoid bacillus is exceptional in not attacking dulcite and rhamnose; that the Salmonella group as a whole is unquestionably derived from a lactose-fermenting ancestry in the great group of which *B. coli* is a typical member. I know of no reply to the suggestion that the sudden or gradual modifications of fermentative activity which have been so far observed are merely atavistic—merely express the reassertion of vestigial ancestral factors, exercised into new vigour. What makes this view plausible is that the nearer a non-fermenting form stands to its fermenting congeners the easier it is to induce it to ferment; the further from them the more difficult. My personal view is that in these cases one has to deal

with something in the nature of "germinal selection". I suggest that a minute fraction of the vital substance of the race still carries the ancestral potentiality; that when placed on the special medium the organism first of all exhausts the ordinary nutritive constituents of the substrate; that then is reached a point at which only those elements of the organism which possess the power to attack the test substance can feed and multiply; so must arise the spurt of secondary growth with the development of a race in which the pristine substance has returned to dominance.

Though loss of fermentative activity has seldom been observed in the laboratory there is no doubt that such loss changes have played an important part in the differentiation of bacterial types. Almost all the numerous Salmonella types which serology has defined differ from one another by failure to ferment, or to ferment fully, one or more of a series of carbohydrates and other substances. Indeed the whole trend of fermentation, in the parasitic groups of intestinal bacilli, has been towards suppression. Here and there we may trace the process in its course—non-gas-forming races of gas-forming types; xylose non-fermenting typhoid bacilli; paratyphoid B bacilli which do not attack inosite; and so on. Gaseous fermentation depends on the ability of the organism to split into H_2 and CO_2 the formic acid produced during acid fermentation. It has been shown that various gas-producing organisms cultured on a medium containing chlor-acetic acid lose in later culture their power to attack formic acid, and with it ability to liberate gas: thus has the evolutionary event been imitated in the laboratory.

Now as regards variations in pathogenicity. As I have already indicated serological roughening of pathogenic species is associated with an abrupt loss of virulence; with return to the normal smooth state virulence reappears.

The apathogenicity of the rough form is a matter of medical and economic importance: it probably in part explains the wane of epidemics. The so-called "rat viruses", bacilli exploited for destruction of rats, furnish an example. By mouse to mouse passage these organisms may be maintained in a state of high virulence in which they kill a high percentage of rats directly infected; but when the attempt is made to convey them from rat to rat their virulence sooner or later decreases and dies away bringing the series to a close; the organism has become rough under the weathering action of antibodies. It would be rash to assert that roughening is the sole cause of loss of bacterial virulence, but it is certainly an important cause.

How far may the virulence of the virulent form be increased? how far may the range of its pathogenicity be widened or changed? how far may non-pathogenic species of bacteria be rendered pathogenic? There are declarations on all these points. It is said that by passage the anthrax bacillus may be rendered virulent for the at first refractory dog; that the pathogenic predilection of the rotlauf bacillus may be transferred, by the same means, from the pig to the rabbit; that by culture within delicate celloidin capsules in the peritoneum of a living host utterly harmless saprophytes such as *B. megatherium* may be guided to pathogenicity; that others such as *B. mycoides*, having been first acclimatised to blood temperature, may be rendered infective by passage through animals sensitised by an injection of the killed organism. These alleged changes have the appearance of direct and heritable adaptations and have usually been interpreted as such. Some or all these claims may be justified, but there is something, perhaps in their remoteness—for most, I think, originated in the earlier days of bacteriology—which raises in the mind a certain

scepticism as to their general validity. Nowadays we speak on the whole less largely than of yore of adaptative changes in bacterial virulence and pathogenicity. One considerable investigation of Salmonella infections in mice led to the conclusion that the virulence of the strain was a fixed quantity, unraised by serial passages and unreduced by years of saprophytic growth. Certain other experiments, stimulated by this untraditional conclusion, suggested that, when parallel series of passages were made from the same strain, a significant augmentation of virulence may occur in some lines and not in others: such irregularity is perhaps more suggestive of chance variations than of gradual direct adaptation.

In treating of virulence we may conveniently include the filterable viruses. If the direct evidence of pathogenic modification in bacteria is somewhat unsatisfactory, that supplied by the viruses is conclusive. An outstanding instance is the alteration of the rabies virus during rabbit passage with increasing virulence for that animal and suspension of its virulence for man. The adaptation of the virus of foot-and-mouth disease to the guinea-pig and small rodents is another well established case of change. By intra-cerebral inoculation of the vaccine (cow-pox) virus in rabbits there has arisen a race with vastly enhanced virulence for these animals—the so-called neuro-vaccine. Examples might be multiplied; but these will suffice. In nature evidence of evolutionary divergence among viruses is not difficult to trace. In the viruses of cow-pox and small-pox we have biological races which while diverging in pathogenicity have maintained their immunological unity; in the races of the foot-and-mouth virus we have the reverse situation.

How virus forms with relative readiness modify their pathogenic propensities to the occasion is hardly clear, and I have no special right to an opinion. Discussion with colleagues engaged in virus studies has raised the suggestion that the virus may actually be in a position to adopt something from its new host which renders it compatible with the environment afforded; that it virtually "makes up" for the part it is to play with the substance of its host and so, as it were, under false pretences gains access to the cells and tissues. When we recall the transformation of the pneumococcus types referred to earlier this fantasy seems to come within the limits of biological possibility.

To return to the bacterial world: it remains to draw what general conclusions we can as to the factors and mode of microbic evolution. Most medical writers who have ventured an opinion on these questions have leaned towards a theory of progressive and heritable adaptation; the frankly Lamarckian view has been sponsored by Adami in particular. It is easy to be impressed by the specialisations and variety of bacterial parasitism and to grasp the simplest of theoretical explanations. But, apart from the fact that the plasticity of bacteria does not seem to be so great as was at one time suggested, a theory of directly adaptative evolution presents difficulties so soon as the assumed instances are viewed in proper perspective. At first glance it is tempting to regard the deadly toxin of the diphtheria bacillus as a special adaptation to parasitic life. But *B. botulinus*, which is not a parasite, produces a poison of the same type and even more intensely lethal; what advantage the organism derives from its terrible product it is difficult to conceive. We are forced to conclude that the toxins as a class are metabolic accidents which may, other things favourable, fit the organism for a parasitic life. Take another case: the European hog cholera bacillus is widely distributed in swine and is almost certainly frequently consumed by man without

untoward result. Occasionally, however, there occur outbreaks of acute gastro-enteritic food poisoning through eating food contaminated with this bacillus, and every now and then there crop up single cases of a paratyphoid nature due to what so far as we can tell is the same organism. We are probably right in believing that the European hog cholera bacillus includes races, some harmless, some harmful to man. But what is of interest is that human infection by this organism has no tendency to spread; it is a cul-de-sac in the dissemination of the organism. We are therefore forced to conclude that pathogenicity for man was developed in the pig and not in the human host.

In the pig, its natural host, the American hog cholera bacillus normally behaves as a harmless saprophyte, only invading the tissues in company with the virus of hog cholera. But of Salmonella bacilli this organism is the most virulent for the rabbit; half a dozen bacilli, introduced subcutaneously, almost invariably prove fatal: yet the organism is unknown as a cause of spontaneous disease of the rabbit; its "adaptation" to the rabbit must have evolved in the porcine host.

The present focus of our researches is on a relatively small number of bacterial species which on account of their pathogenicity demand first attention. These organisms, specialised in parasitism, express in their own way the process of secondary simplification which is characteristic of parasitism in higher forms, and I have indicated how readily we may discover, and even in some cases produce, the racial material on which phylogenetic reduction of characters may depend. Possibly when we attack less specialised saprophytic groups of bacteria by the detailed methods we have elaborated for parasitic forms we may hope to discover more definite evidence of progressive evolution on the wing.

We cannot but be struck by the fact that bacterial species—especially those mapped out by serological means—are as sharp as any. Modern bacteriology sees little of the indefinite and blurred groups of varieties admitted in the past. The whole picture is vastly more suggestive of discontinuous variation than of any continuous process. It is noteworthy that while the pathogenic groups of bacteria are as a rule relatively simple—represented by one, two or three types—or perhaps a few dozen—their nearest non-pathogenic congeners often present an array of forms enough to break the stoutest taxonomist heart; sometimes the types appear to be as numerous as the strains we isolate. The conclusion must be that the conditions of parasitic life either discourage variation or blot out the vast majority of variants. The second line of explanation is, I think, the more probable.

In the bacterial world the parasitic species are a mere handful; the parasitic genera still fewer and it is possible that in several cases two or more of these genera are the product of a single parasitic shoot. There is no general bacterial offensive against higher nature; existing pathogenicity has evolved from a few definite points. There is nothing incompatible with the view that pathogenicity is a fortuitous property. Though the direct evidence may be trivial the circumstantial evidence is to the effect that bacterial divergence is achieved by chance non-adaptative variations and by the sudden or gradual disappearance of factors unpreserved by selection and use.

One last point—the bacteriophage: d'Hérelle, the discoverer, has pointed to the bacteriophage as an active agent in producing bacterial mutations; and it is true that a considerable selection of variant forms appear in partially lysed cultures. There is, however, as yet no evidence that these "mutants" differ in any positive particular

from the parent strain or transgress the limits of ordinary serological variation. Bordet, discarding the parasitic theory of the bacteriophage, sees in the "principle of transmissible autolysis" a normal bacterial ferment run amok. In seeking the origin of microbic transformations we must at least consider the possibility that some such stimulant of metabolic unrest may play a part in disturbing the stability of the idioplasm.

V. BIOLOGICAL RACES IN SEED-BEARING PLANTS AND THEIR SIGNIFICANCE IN EVOLUTION.

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Introduction. Terms, etc.

I have been asked to introduce the subject of Biological Races in Seed-Bearing Plants and their Relation to Evolution. An initial difficulty has been to decide exactly what should be and what should not be included under the phrase "biological races." The term "race" is one of those unfortunate terms to which many diverse meanings have been attached by different authors. For that reason one is perhaps justified in retaining it for use in a rather vague sense, without at any rate attaching an exact taxonomic meaning to it. In perusing a fair amount of literature on the cryptogams, with reference to the subject of to-day's discussion, I have not found any marked agreement as to the exact use of the phrase "biological races" and the various other phrases more or less synonymous with it. The underlying idea is usually that certain groups of individuals differ from other groups of the same taxonomic or morphological species or even variety in physiological or biological characters, often in these alone but sometimes also in morphological characters. The importance of biological races in the fungi and bacteria, lies, of course, in the fact that if these plants did not exist most pathologists would be unemployed. There has not been the same incentive to study physiological races in the Spermatophyta, with some exceptions of which the most important is the converse of the pathologist's studies, namely, susceptibility, resistance, and immunity in the host, when this is a seed-bearing plant.

Two matters, not unconnected, stand out in studies on biological races in the higher plants: firstly, that a number of different physiological or biological characters have been definitely shown to have a genetic basis and, secondly, that "races" differing in biological or physiological characters frequently also differ in morphological characters. In other words, there are relatively few examples of "biological races" in the strictest sense and even those recorded are often suspect to the taxonomists. It may be suggested that one reason for this is that, as compared with the lower unicellular, filamentous, and coenocytic plants, the seed-bearing plants have a greater range of organs and therefore many more obvious morphological characters which can be used to differentiate taxonomic units. For this reason I have no scruples in dealing with characters as much as with races.

Susceptibility, resistance, and immunity.

It is well known that different races and varieties of the same taxonomic species of seed-bearing plant often behave differently with regard to fungous, bacterial, or animal attack. Many of the facts are well known, and though there has been much

dispute as to the morphological or physiological "causes" of immunity or its converse, the evidence is conclusive that there is often a genetic basis for the behaviour empirically found. The gene or genes essentially responsible for immunity or susceptibility are not usually linked with genes responsible for morphological characters throughout an entire species. That is to say, re-combinations of physiological and morphological characters can be made. In this way immune varieties, in which immunity is associated with other desirable characters, have been made in certain important crops. Wheats resistant to various rusts and potatoes immune to wart disease are amongst the numerous well-known examples. It appears that the inheritance of immunity is sometimes explicable on an ordinary monohybrid basis, sometimes on a modified dihybrid basis. Moreover, even in resistance to the same pathological organism, as *Melampsora lini* on flax, ratios approximating to both 3 : 1 and 15 : 1 have been obtained (1). Yet again, the gene or genes responsible for resistance, even to the same species of fungus, are sometimes dominant, sometimes recessive, in different races of host, and sometimes blending is apparent in F_1, indicating that such genes differ in their nature (2). Finally, the plasticity of the host is often an important factor, resistance varying with different environmental conditions, and, since compatibility depends also upon the inherent constitution, including plasticity, of the parasite, a very large number of phenotypic groups result.

Instances of selection of biological races by insects have been recorded (6). Molz (27) has suggested that the resistance of African cacao to the attacks of the larvae of *Ephestria elutella* is due to the richness of the bark in tannin matter, which is present in only small quantities in the more susceptible Guatemalan and Venezuelan varieties. Prell (25) has attributed the immunity of certain pines from the attacks of the nun moth (*Liparis monacha*) to the presence, in the leaves, of about ½ per cent. of turpentine, which is absent from the leaves of other pines. The possibility of the preferences of insects being of importance is indicated by some research on *Hypericum* which we did in connection with the biologic control of St John's Wort in Australasia. Briefly, we showed that the plant which has caused so much trouble in parts of New South Wales, Victoria, and South Australia is a Mediterranean variety with narrower inrolled leaves—var. *angustifolium* DC.—while that in New Zealand is indistinguishable from the ordinary British plant. It remains to be seen if the insects introduced successfully attack both varieties in their adopted homes.

The use of plants as sources of food by higher mammals sometimes enables us to distinguish biological races. Certainly some animals eat anything. In Macedonia I have seen the scraggy sheep and goats eating a dead sheep and in Sofia I have seen a goat tearing down and eating paper advertisements. A few such animals might be useful here! Often, however, sheep, and still more cattle, are decidedly selective. The best instance I know was given me by Mr C. A. Smith of Pretoria. In the Fauresmith district of the Orange Free State one of the best forage plants is *Salsola glabrescens* the young shoots of which are eaten by sheep and goats. The local farmers distinguish by vernacular names two races which the botanist can only identify by the one having the young branches red and the other having them white. The sheep eat only the red-branched plant when both are present and will only nibble at the white one when the red has been all eaten off.

Spermatophytic parasites.

The closest comparison amongst seed-bearing plants with biological races in the lower plants must naturally be sought in the relatively few parasitic genera and species of Spermatophytes. Let us consider briefly the Orobanchaceae (3), an entirely parasitic family related to the Scrophulariaceae. Most of them live on herbaceous seed-plants and die down with the latter, a few parasitise woody hosts. Monocotyledons are only recorded as host plants for *Aeginetia* and *Christisonia* and the occurrence of *Orobanche* on vascular Cryptogams is doubtful. Within the genus *Orobanche* we find some species with a wide range of hosts: *O. minor* has eighty-nine recorded hosts, *O. mutelii* fifty-five, *O. nana* fifty. On the other hand some are limited to species of only one family, especially to Leguminosae, Labiatae, and Umbelliferae, or to one genus, as *Artemisia* or *Centaurea*. In nature *Orobanche laserpitii-sileris* is limited to *Laserpitium siler* and *O. hederae* to the ivy. In this country *Orobanche major* was recorded only on *Centaurea scabiosa* till last year we found two fine plants on knapweeds of the *nigra* group in the *Centaurea* plots at Potterne. Although, then, we have no knowledge of purely biological races, indistinguishable by morphological characters from one another, in the Orobanchaceae, we do find various grades of specialisation of parasitism in the family, and purely biological races might be found if sought. It is interesting to note that increasing specialisation can be traced through the semi-parasitic Scrophulariaceae to the completely parasitic Orobanchaceae, and that this is, in general, correlated with a decreased range of hosts (4). Specialisation also appears to be associated in some degree with an increased output of seeds—the risk of no seedlings finding a suitable host being thus reduced. Very similar conclusions are reached in studies on *Cuscuta* (Convolvulaceae), one recalls *Cuscuta trifolii*, *Cytinus*, *Balanophora* and other parasitic genera. The three varieties, or whatever they be considered, of *Viscum* are biological rather than morphological races limited to distinct series of hosts (5).

Edaphic limitations.

The majority of seed-bearing plants are rooted in the soil from which they obtain their mineral food in solution. It is known that taxonomically allied but morphologically distinct species of the same genus frequently show different soil preferences, but there has been little research on the more subtle differences of races of one species which show edaphic limitations. A considerable number of plants (species and varieties) limited to soils derived from serpentine rocks are only slightly different morphologically from their congeners. Examples of species entirely or almost entirely restricted to a serpentine substratum in the Balkan Peninsula are: *Halacsya sendtneri*, *Potentilla visianii*, *Cerastium alsinifolium*, *Euphorbia gregersonii*, *E. serpentini*, *Ruta* (*Haplophyllum*) *boisserianum*, *Genista hassertiana*, *Viola dukadjinica* and *Sedum serpentini*. A large number of "sub-species," "varieties" and "forms" have also been described as limited to serpentine under natural conditions (7). Even better known are the examples of species or varieties limited to calcareous rocks. It is, however, worth recalling that the edaphic limitation or preference of a species or variety in one country may not be the same as in another country with different climatic conditions (8).

Drought resistance.

Another basis for the distinction of biological races is drought resistance. Maximoff (9) has urged that the real test of xerophytism is recovery from prolonged wilting. In areas where dry farming is an economic necessity the selection of races of crop plants capable of surviving periods of drought is all important. One may mention the extension of the Australian wheat belt through the results obtained at the Waite Institute, South Australia, the breeding or selection of more drought resistant "races" of maize in America, *Sorghum* in India, *Lolium* and *Festuca* in New Zealand, and *Linum* in India. Selection for cold and drought resistance has enabled lucerne to be grown successfully much farther north in the U.S.A. and in Canada than was formerly possible.

Cold resistance.

Resistance to cold or heat is intimately connected with distribution in altitude and latitude. The physiology of frost resistance has been studied in some detail (10, 11) and Lidforss, for example, has shown that all terrestrial winter-green seed-bearing plants in the Swedish flora contain quantities of sugar but are free from starch in the winter, though the same leaves contain abundant starch in summer. *Acer negundo* from Virginia is killed in the winter in the north-western states and in Manitoba, while the local forms of box elder indistinguishable from the southern by morphological characters, are perfectly hardy. In northern Russia *Acer negundo* from seed from near St Louis proved tender in the north, later seed from much farther north has been tested and found hardy. The black walnut and a number of conifers have races varying in hardiness. The deciduous or evergreen habit is partly correlated with temperature. At Kew several species are grown which have two races, the one evergreen the other deciduous, as *Quercus castanifolia*, *Cedrus libani* and *C. atlantica*. A very striking example is furnished by *Magnolia virginiana* var. *glauca*. Near Museum No. 1 at Kew there are two bushes of this variety, the one from the north-east United States sheds its leaves in the autumn, the other from more southern States retains its leaves till the new season's buds burst in spring. The origin of high mountain plants has frequently been discussed, especially with reference to those of the European Alps, but the subject is less complex in parts of the Mediterranean Region. In Greece and Crete, for example, the vast majority of the high mountain plants are closely related to lowland species with which they are altitudinally vicarious. A detailed study of many of these has convinced me that selection of biological characters suited to the different environments has been a main cause of the origin of these vicarious species (8).

Phenology.

Very closely connected with resistance to cold or heat are variations in phenological data. One of the main criticisms of the phenological research edited by a committee of the Royal Meteorological Society is that little or no attention has been paid by the committee, to judge from their latest published report, to the occurrence of races with inherent differences in seasonal behaviour within the same taxonomic species. To quote an example from a published notice (12): "The inclusion of black knapweed in the list of plants is open to considerable criticism. Recent research has shown that *Centaurea nigra*, in the broad sense, is an extremely polymorphic species,

that not only are there many varieties, but that, especially in the south of England, hybrid populations between it and *C. jacea* are not uncommon. Moreover, at the Potterne Research Station and at Kew, earlier and later flowering races have been obtained from wild material, precocity of flowering being, for some races at least, a definitely inherent character. It is stated that the black knapweed has given less satisfactory results than the other species and the reason suggested is that observers have confused it with *C. scabiosa*. If this is so, there is little chance that *C. jacea*, *C. nemoralis* and hybrid populations have been distinguished and the value of the massed records of nearly 500 observers can only be small." As indicated in this paragraph we have selected from wild populations races with markedly different flowering periods, and transplant experiments have shown that these biological differences are retained on six different soils and at both Kew and Potterne. The behaviour of horse chestnuts has interested me. A particular tree at Richmond is always in leaf and then in flower earlier than any others in the neighbourhood. Every year for over twenty years of my own observation, except for the war years, of two horse chestnuts side by side near the Main Gate at Kew the one on the east burst its buds, flowered and shed its leaves ten days earlier than the tree close to it on the west. Unfortunately one of the trees has now been destroyed in a gale.

Early and late varieties of many important crops, as potatoes, tomatoes, and peas, are well known. *Cannabis sativa* var. *praecox* is an early variety of the common hemp, and occurs as a pure crop, or in admixtures in the northern districts of Russia (13). The results obtained by the geographical sowings commenced in 1923 at 115 different stations in the U.S.S.R. already show what very different phenological characters are possessed by the 185 winter and spring varieties of cultivated plants which are being experimented with in so-called pure lines (14).

It is important for us to note that several well-known genera have spring- and autumn-flowering species, some of which can be paired on morphological grounds—an autumn-flowering species being very closely related, sometimes scarcely distinguishable, from a spring-flowering one. *Colchicum*, *Crocus*, *Scilla* and *Taraxacum* come to mind. If community of morphological characters is a measure of phylogenetic relationships, one must conclude that many an autumn-flowering species has had a not far distant common ancestor with a spring-flowering species.

Wettstein (15) for the genera *Gentiana* and *Euphrasia* and Sterneck (16) for the genus *Alectorolophus* have described a "seasonal dimorphism" by which species have split into two—a spring-flowering and an autumn-flowering. Moreover, these authors say definitely that there is a tendency for convergence in morphological characters of the different spring- and autumn-flowering segregates respectively, so that several species flowering in the autumn may come to have more superficial resemblance one with another than any one has to its real spring-flowering partner.

Seed behaviour.

A brief reminder may be given of the many biological characters concerned with seeds and their germination. The subject has been excellently discussed by Prof. Salisbury (17). One example, additional to those given by him, has resulted from the British Ecological Society's Transplant Experiments at Potterne (18). *Silene vulgaris* seeds germinate in the spring. Those of *S. maritima* have two discontinuous periods of germination, in the autumn immediately after ripening and in the spring of the

next year. The interesting feature is that autumn germination may take place in the capsules and the radicles then grow through the persistent bladdery calyx. It is probable that this is one biological character which enables the species, unlike its closely related congener, to establish itself on shingle beaches, the calyces preventing the seeds from slipping deep into the crevices where the seedlings would have little chance of survival. Intensive work for six years has shown that the two species are very closely related in every way and that the differential characters of greatest biological importance are physiological or morpho-physiological.

Chemical differences.

Without doubt many of the biological characters already referred to could be shown to have a chemical basis. We have, however, a few definite examples which have been studied in some detail. Penfold and Morrison (19) investigated the oil of *Eucalyptus dives* and recorded a series of plants, within the one botanical species, containing varying proportions of piperitone, phellandrene, and cineol. Although the authors made a type and three varieties on the chemical constitution of the oil there are intermediates. On the other hand ecological conditions can scarcely account for the differences, since "the type" and "varieties" have been found growing together.

Oils which are the main food reserve of many seeds appear to be partly dependent in quantity and quality on environmental conditions and partly on genetic constitution (20, 21, 22).

"Strength" in wheat, *i.e.* in bread-making the production of an open texture and good volume, is determined chiefly by the physical properties of the gluten the flour contains. Work at Cambridge has shown that strength is a characteristic of independent gene inheritance (23).

That *Solanum nigrum* must exist in several biological races, differing chemically, as well as in a number of morphological varieties, is evident from the vogue of the "Wonder Berry" a few years before the war. It was impossible to separate morphologically the plants distributed, originally from America, under this name, from the common weed of this country—the black nightshade.

Armstrong, Armstrong and Horton (26) have shown that the "form" of *Lotus corniculatus* common in south Britain contains both a cyanophoric glucoside and the correlated enzyme, while in Scotland and Norway a "form" prevails which is "rich" in enzyme but contains mere traces of the glucoside. A third "form," much rarer, exists in which the amount of enzyme is also very small. Apparently no constant morphological distinctions could be detected between the forms. The same authors also give evidence of similar differences within *Trifolium repens*, and it is well known that races of *Sorghum* differ in yield of HCN.

The variations in quality and quantity of products from a great many economic plants is a matter of considerable importance. One may merely mention by name as examples: sugar cane, tobacco, camphor and *Hevea.*

As the Director of Kew indicated at Bristol last year the botanist and chemist have much team work to do (6).

Competition.

The importance of biological characters in the establishment of seedlings in some habitats has already been suggested. Even if competition, in the broadest sense, is most severe in young stages of development, it never completely ceases. Sukatschew (24),

experimenting with dandelions, found that "races *A*, *B* and *C*" from the same lawn were distinguishable in "fitness" or "vigour" when grown in pure and mixed cultures. After two years, 27 per cent. of *A*, 49 per cent. of *B* and 24 per cent. of *C* survived in the pure cultures and 23 per cent. of *A*, 20 per cent. of *B* and 58 per cent. of *C* in the mixed cultures. Vigour, in these dandelions, is fitness in a given environment, and is mainly determined by physiological causes. No one could have predicted the above results from an examination of the morphology of *A*, *B* and *C*.

Conclusions.

It is hoped that enough has been said to prove that biological characters of various kinds are widespread in seed-bearing plants, have, at least often, a genetic basis, and may serve to distinguish, without or together with accompanying morphological characters, biological races or varieties. An attempt has been made to indicate the wide range of these biological characters rather than to deal exhaustively with any one kind. No doubt, those taking part in the discussion will give numerous other examples and will suggest additions to the kinds.

We turn to a brief consideration of the significance of biological races and characters in evolution.

With regard to immunity and susceptibility, debate as to which came first, host or parasite, might lead to a good show of wit. While most mutations of observed origin are recessive, dominant mutations are not unknown. Moreover, immunity is sometimes dominant, sometimes recessive, and infection or its failure depends on characters of both host and parasite. Environment also plays a part, sometimes a predominant part. All one can say is that survival for a seed-bearing plant, within a given area, under a given set of conditions, depends on immunity or a sufficient degree of resistance to any parasite which could otherwise prevent seeding. That immune or resistant "races" might be selected for survival in nature owing to the attack of a parasite seems very probable, but I have no example to quote.

In seed plants, parasitic on other seed plants, we have some examples of high specialisation, and it may not be without significance that in general the species or varieties more limited in their hosts are less common and have a narrower geographical distribution than species which, like *Orobanche minor*, are less particular. Over-specialisation is sometimes less likely to lead to survival, and certainly to further evolution, than a more accommodating kind of parasitism.

It is difficult to generalise about races or characters with edaphic limitations, because the Potterne Transplant experiments (18) are showing how varied is the behaviour of different species and how numerous are the causal factors involved. The experiments have not yet reached the stage where evidence for or against genic modification can be expected, but the importance of biological characters as indicated by survival on one or more soils and death on others, or by plasticity leading to vigorous or weak growth, has already become evident. Considering these experiments one cannot help feeling that a geographical factor involving the chance of a new variant being able to reach a locality with a suitable environment may often be the controlling factor in its survival and spread.

Reactions to drought and frost and phenological variations obviously set varied limits to geographical distribution and have thus a bearing on isolation and possi-

bilities of hybridisation, both of which, in their turn, have been involved in producing the kinds of existing plants and animals, *i.e.* in evolution.

Races of plants differing from one another in biological characters which have no apparent survival value are exemplified by some of the examples quoted, notably by those with certain different chemical constituents. It is interesting to recall that many morphological characters of allied species or varieties have no obvious survival value and cannot be accounted for, at least with our present knowledge, by natural selection.

Sergius Ivanov, after dealing more especially with seed oils, states that "physiological mutants" do not exist, and also that physiological characters are less dependent than the morphological on external conditions. The physiological characters strive to preserve the species unchanged, the morphological characters change it and produce new species (20). Both of these conclusions are open to doubt. We have seen that certain physiological characters have a definite genic inheritance and it is a working hypothesis that they have had a mutational origin. That physiological characters are to some extent, and sometimes to a considerable extent, plastic under varying environmental conditions is shown by some of the recent Russian work on cultivated plants.

In studying biological races and their significance in evolution botanists dealing with seed plants will have to combine their methods, which at present are far too isolated. This will involve more team work. The herbarium and museum taxonomist can confirm taxonomic identifications and contribute data on the presence or absence of morphological characters separating races with different biological characters. The cultivator and geneticist are obviously of first importance. Laboratory studies will involve the anatomist, cytologist and chemist. Extensive field work of an ecological and phytogeographical nature can alone interpret the intensive researches in such a way as to indicate what actually happens and has happened in nature.

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In contributing to the general discussion[1] Prof. MacBride said that he had listened with the greatest interest to the papers of Dr Thorpe and Dr Goodey, in which evidence had been produced that allied races in many cases differed from one another in habits but hardly at all in structure. They had also produced evidence that habits acquired by one generation owing to exposure to a special set of circumstances could be handed on to subsequent generations. This in his opinion was a point of fundamental importance in considering the causes of evolution. The readers of the papers had pointed out that it might be held that their results supported Lamarckism or the doctrine of the inheritance of acquired qualities, but that in their opinion an alternative explanation was possible, viz. that the transmitted habits might not be cases of direct inheritance but of memory. Now the introduction of the word "memory" was apt to cause some confusion of thought, because this word was used in ordinary speech in two totally different senses. It might mean the reproduction in consciousness of a long past experience, and in this sense it must be applied to the lower animals with extreme caution, or it might mean the revival of an acquired habit. When we learned tennis in youth, and during a long period in middle life were unable to find opportunities for playing the game and then in later life returned to circumstances in which the game could be indulged in, much of our early skill would be lost through disuse, but a certain portion remained, and by further use rapidly attained its former dimensions—as we expressed it our skill came back to us. This persistence of skill might be termed memory, but if so it should be distinguished as *habitudinal memory* from true *psychical memory*. Now habitudinal memory was simply Lamarckism, as the following quotations from the *Philosophie Zoologique* (Elliot's translation) would prove: (1) "The environment affects the shape and organisation of animals, but whatever environment may do, *it does not work any direct modification whatever in the shape and organisation of animals*, but great alterations in the environment of animals lead to great alterations in their needs and these alterations in their needs lead to alterations in their activities. Now if the new needs become permanent the animals then adopt NEW HABITS which last as long as the needs that evolved them." And again (2) "In every animal which has not passed the limit of its development, a more frequent and continuous *use* of any organ strengthens, develops and enlarges that organ and gives it a power proportional to the length of time it has been so used, whilst the permanent DISUSE of any organ imperceptibly weakens and deteriorates it and progressively diminishes its functional capacity until it finally disappears." And (3) "All the acquisitions or losses wrought by nature on

[1] Following Dr Goodey's paper.

individuals through the influence of the environment in which their race has *long* been placed, and hence by the predominant use or disuse of any organ, all these are preserved by reproduction to the new individuals which arise, provided that the acquired modifications are common to both sexes or at least to the individuals which produce the young."

According then to Lamarck change in the environment leads to the production of new situations which demand new activities on the part of animals—these activities become habits. Habits long persisted in are transmitted to offspring and eventually give rise to changed structures.

Dr Thorpe had alluded to the hereditary transmission of acquired habits by the saw-fly *Pontania salicis*, discovered by Heslop Harrison as possibly to be explained by memory. Prof. MacBride took a great interest in this paper as he had had the honour of communicating it to the Royal Society. Harrison forced this saw-fly to change the species of willow on which it laid its eggs. When the new habits had persisted for five years, he introduced the original species of willow, but the saw-fly persisted in laying its eggs in the new species. Now in what did the supposed memory of the saw-fly consist? If it meant a psychical revival of the taste of the plant on which it had fed whilst a larva, then this habit ought to be formed at once and the descendants of that saw-fly ought after one generation always to go to the species of willow on which their mother had laid them, but it took Harrison five years to stamp the new habit in. If it meant simply that the tendency to go to a particular species of willow became more and more engrained in the constitution as generations succeeded one another then this was pure Lamarckism. The psychical explanation would not explain the case mentioned by Dr Goodey in which the head louse of man which has slight structural differences from the body louse, was transformed into the body louse in three or four generations by rearing it in a dark box. Nor will it explain the results obtained by Dürkhen who reared the caterpillars of the common white butterfly under orange-coloured glass and found that 66 per cent. of the pupae were green instead of the normal grayish white colour. This was due to the inhibition of the formation of the normal pigment of the pupal skin which allowed the green blood to shine through when these pupae gave rise to imagines which mated and laid eggs and when the resulting caterpillars were again reared under orange glass they gave rise to 95 per cent. of green pupae. When the caterpillars of this generation were reared under ordinary glass 34 per cent. of them were green. Controls which had not been subjected to orange light gave only 4 per cent. green.

Just as the production of one genuine ghost would prove the existence of a future life, so the demonstration of one genuine case of the inheritance of acquired habit would prove the validity of the Lamarckian principle and afford an explanation of the whole of evolution. In Prof. MacBride's judgment this latter feat had been successfully accomplished.

Certain objections to Lamarckism might now be dealt with. It was often urged that what appeared to be Lamarckian inheritance might be due to natural selection. This objection was due to a confusion of thought. Natural selection *per se* explained nothing, it only meant that "the survivors survive." No evolution at all could take place without variation, *i.e.* change in the hereditary powers. What was covertly assumed in the explanation by natural selection was that changes in the hereditary powers in *all directions* were constantly going on, for no reason whatever, *i.e.* by

chance, and one of them which happened to fit the environment persisted and was "naturally selected." Of this "chance" production of variations in all directions there was no proof and the pure line experiments seemed definitely to negative it. But it might be urged that the experience of cattle breeders and of gardeners afforded evidence of the occurrence of most varied crops of "mutations" with no conceivable relation to function or environment. This must be admitted. But two things were forgotten, first that these mutations arising under conditions of domestication (however useful to man) were all physiological weaklings as compared to the type and would never survive if exposed to competition in the open field; secondly, that the experiments by X-rays on the eggs of *Drosophila* by Muller proved that they were produced by *injury* to the germ in an early stage of its development. It might be added that when domestic animals ran wild in a country where their natural enemies were absent, they revert to the wild type, as in the case of the domestic pigs released by Captain Cook in New Zealand. Germ injury had nothing in common with functional variation.

Finally it was sometimes urged that if there were inheritance of acquired characters, such a character should be inherited by the offspring in full force even when the environment had been totally changed. But this was a total misconception of the Lamarckian principle. An altered "character" was the result of an altered intensity of growth: if this alteration were due to the response of the animal to a new environment, then when the offspring were replaced in the old environment, this should tend to change the mode of growth back to the typical form. If there was some carry-over of the effects produced in one generation to the next—and this was all that Lamarckism demanded—the utmost that we could expect to see, and what as a matter of fact we found was a *lag* in the assumption by the offspring of typical characters when they were replaced in the old environment, some trace of the exposure of the parents to the new conditions still remaining, and an *intensification* of the alteration of the characters due to the new environment when the children continued to live in the new environment in which their parents had been placed.

To sum up, the objections to Lamarckism resolve themselves into the determination to neglect the time factor: a factor on which Lamarck laid especial stress—and "time pays no attention to experiments which leave her out of account."

PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS. II

ORDINARY MEETING held on Friday, March 20th, at 2.30 p.m. in the Botanical Lecture Theatre of the Imperial College of Science and Technology, London. The Chair was taken by the President, Dr A. D. IMMS, F.R.S.

The following papers were read:[1]

I. Retrospects and Prospects of the Economic Application of Microbiology. By Dr A. C. THAYSEN.

II. A general Review of the Microbiology of Cellulose and its Associated Compounds. By Mr H. J. BUNKER.

I. RETROSPECTS AND PROSPECTS OF THE ECONOMIC APPLICATION OF MICROBIOLOGY.

By A. C. THAYSEN.

(*Bacteriological Laboratory, R.N. Cordite Factory, Holton Heath.*)

IT must be about a year ago that we listened at this place to Prof. Stenhouse Williams' interesting account of the activities of the Dairy Research Institute at Reading, an account which demonstrated the importance of the study of the various micro-organisms which are present in milk, not least in order to ensure the introduction into butter and cheese manufacture of exactly those types, and no others, which are required for the flavouring and ripening of these foods.

A practical application of bacteriology as distinct from a study of bacteriology is now being attempted also in soil science where, in this country, the Rothamsted Institute has done valuable pioneer work in raising the fertility of a soil by the introduction of nitrogen-fixing micro-organisms and in the production of synthetic farmyard manures.

Of course there are other applications of bacteria and much older ones. Take medical science, for instance, where the use of micro-organisms in the preparation of vaccines and antisera, not to mention in the artificial inoculation with malaria protozoa, has been of great value. And then there is the oldest application of all, and some people might think the most important of all, the use of micro-organisms in the distillery and in the brewery. Though important and diverse all these applications strike one nevertheless as rather accidental; not the outcome of a natural evolution of the science of bacteriology, but rather as the expression of a need felt by various human spheres of interest to get the upper hand in cases where fate had brought them face to face with the consequence of the most striking characteristic of micro-organisms—their amazing power of reproduction.

[1] The authors desire to express their thanks to the Admiralty and to the Department of Scientific and Industrial Research for permission to read these papers.

Now, these spheres of interest are by no means always occupied with biology as such, they approach the study of micro-organisms from their specific points of view which may be those of a medical man, or of a chemist, or even of a civil engineer. The result has been that to-day you can distinguish three or four different, and more or less independent, types of bacteriology rather than a single one. You talk about medical bacteriology, or about soil bacteriology, or sewage disposal engineering, or brewing chemistry but not about bacteriology, or better still microbiology, as applied to medicine, agriculture, etc.

This difference in expression is really of much greater significance than would appear at first sight, for it brings into sharp relief some serious errors made by past generations of bacteriologists. Through these errors the study of microbiology has become subdivided into a number of water-tight compartments, and the workers in the different sections have lost contact not only with each other, if ever there was such contact, but with the general biological aspects of their science which should be common to them all. Take a medical bacteriologist for instance—I am not thinking specifically of those in this or in any other country—how often is he interested in the biology of the micro-organisms with which he works? And the biochemist, who for some reason or other, selects micro-organisms for his study of one aspect or another of the chemistry of life; he will very often, and quite frankly, admit that the general biological properties of the "bugs" with which he is dealing, fail to interest him so long as he is reasonably certain that these properties do not affect the results of his experiments. And these results? Well, he will express them in the language of the chemist and often therefore in a form which the microbiologist with a more biological, or perhaps a medical, training finds it difficult to follow and to appreciate. The valuable results obtained by the chemist are often overlooked, therefore, by the people whom they were meant to benefit and the biochemical aspect of microbiology suffers in consequence.

Let me endeavour to explain in somewhat greater detail, and by means of a few examples, how the excessive specialisation now prevailing has hampered progress. I have chosen for the purpose three different aspects of microbiology, one dependent on a knowledge of the general biological properties of micro-organisms, another on a knowledge of the chemical activities of micro-organisms and a third on their bio-physical properties.

It may have struck you that, in the short time during which I have had the honour of addressing you, I have referred to bacteriology and to microbiology as synonyms of the same science. The extensive adoption of the use of synonyms is, I am sorry to say, a very prevalent fault in microbiological nomenclature to-day and has its root, I venture to suggest, in the almost complete ignorance of biology which many micro-biologists unblushingly profess. You have a very striking example of this in the discussion which has proceeded now for many years on the question of the variability of micro-organisms. It started, this discussion, in 1907 when Massini published his observations on what he chose to call "bacterial mutations," and it has been going on ever since, sometimes under the name of mutations, sometimes under that of functional adaptations, sometimes simply under the general term of variations.

What Massini observed was this. He discovered by chance, on a culture medium containing lactose and decoloured fuchsin, the colonies of a bacterium which, when they had remained in contact with the medium for a certain number of days, showed

very characteristic red-coloured extensions. When he took material from these extrusions he could produce with it red-coloured, that is to say lactose-fermenting, colonies on his medium. The bacteria composing these red colonies were identical in every way with the original ones except in their capacity for fermenting lactose. Since the red colonies arose suddenly and without any apparent artificial inducement, Massini, who must have read de Vries' work on mutation as some people read the Bible, decided to describe the occurrence as a mutation, and as such it became known and discussed among microbiologists who, shall we say, had overlooked that the reproduction of bacteria is not such that it can give rise to mutations which, as you remember, are intimately connected with the development of the chromosomes of the egg cell.

For some time the chief feature of bacterial mutations was thought to be the sudden appearance of secondary colonies in a culture. But after a while it was found that what had happened in Massini's and in all other bacterial "mutations" was that some of the cells in the mother colony had been in close contact with a carbohydrate in the medium and had acquired the property of fermenting this carbohydrate, thus opening up for themselves a new source of energy which allowed them to grow at a faster rate than the main colony and therefore to expand beyond it. It was not a mutation, therefore, but a functional adaptation of some cells of the mother colony to the surrounding medium, an adaptation which could be followed in detail by altered technique. But the name "bacterial mutation" remained in microbiological terminology, and is there to-day.

You may say that this is a rather trifling matter and hardly worth dwelling upon. Yes, perhaps it would be if it were not for the fact that it is typical of the attitude of many microbiologists towards the exploration of the general biological characters of micro-organisms and that, for this reason, it is met with frequently in the much more important question of systematic microbiology. Here we come to a field where every microbiologist seems to have been allowed free and unfettered scope for his own fancies, quite regardless in many cases of biological principles. Take for instance the description and naming of the sorbose bacterium. Bertrand observed that this particular organism was able to convert sorbitol, present in the juice of mountain ash berries, into the carbohydrate sorbose; for this reason he named the organism the sorbose bacterium, without apparently making an effort to see whether it possessed any relationship to other known types. Now, sorbitol and sorbose are not substances which frequently come into the hands of microbiologists and it is little wonder, therefore, that nobody before Bertrand had observed micro-organisms which could convert sorbitol. What many had observed was, that acetic acid bacteria can convert another alcohol—ethyl alcohol—into acetic acid. But that there should be a connection between this reaction and the conversion of sorbitol did not, apparently, interest either Bertrand or anyone else at the time, though Bertand's organism was identical with a well-known acetic acid bacterium in respect of both its morphological and its biological characters.

There are countless similar cases on record, that of *Citromyces*, for instance, so called by Wehmer, because this fungus is capable of converting carbohydrates into citric acid, a characteristic which we now know to be possessed by two large groups of hyphomycetes, the *Penicillia* and the *Aspergilli*. Here again, the "supposed to be" new species, the *Citromyces*, was named without sufficient exploration of its

morphological and general biological characteristics, and merely because it had not yet at the time been observed that the formation of citric acid is a normal feature of the metabolism of all, or most, *Penicillia* and *Aspergilli*. A more recent case is that of Hutchinson's *Spirochaeta cytophaga*, a cellulose-decomposing organism, which everybody is agreed is not a *Spirochaeta* and which, therefore, in the course of its brief span of popularity has had quite a number of names bestowed upon it, most recently by Winogradsky who has made it a type species of a whole group of soil organisms. Here again, I feel convinced, though I express in this respect but a personal opinion, that the organism has been misrepresented because it has been investigated so far only from the point of view of one of its characteristics, its power of destroying cellulose. Its general biological properties have not yet been studied, and it seems to me that for this reason it is unwise to attempt to build up around it at present a complicated taxonomical structure.

The examples which I have given show, I think, that the principles underlying the existing methods of naming and describing micro-organisms are faulty, that too great liberty is given to the fancies of the individual worker with the result that many micro-organisms, I would venture to suggest even the greatest number of existing micro-organisms, remain unrecognised though they may have been already described by one, perhaps sometimes by several workers. Even from a purely practical point of view this is regrettable because it leaves us ignorant in very many cases of the possibilities for the application of microbiology. Take for instance such a group of micro-organisms as the hemicellulose-destroying bacteria. In very many cases these types have been known for years and have been described in the literature—either because they grew on ordinary media in a manner resembling the development of pathogenic forms such as the tetanus bacillus, or for some other comparatively trifling reason. But their true function in nature has remained practically unexplored. And yet this group possesses, in principle at least, potentialities which, if properly studied, might rank the hemicellulose destroyers with yeasts as regards economic importance.

Let me turn now to the two other examples which I have chosen to illustrate my contention that we lack co-ordination in microbiology to-day, that we have been pulling in different directions so to speak and neglected others, and that for these reasons we have hampered progress.

Take the utilisation of carbohydrates by micro-organisms. Here most of us have been content in the past, and are apparently content to-day, to leave matters at establishing whether an organism we are studying produces either gas or acids or perhaps both, when grown in a medium to which a given carbohydrate has been added. Few of us take the trouble to establish the nature of this gas or of this acid, and yet microbiologists who possess special chemical knowledge have long since shown that these products of carbohydrate decomposition are of the greatest importance for the interpretation of the relationship of a very large number of micro-organisms and for the understanding of many apparently unconnected phenomena. Let me recall in this connection the production of capsules and of mucus by various micro-organisms. Without a knowledge of the metabolism of carbohydrates this phenomenon is exceedingly difficult to interpret, as witnessed by a perusal of existing literature. It has quite generally been regarded as a property of sufficient distinction to justify placing organisms which possess it in a class by themselves, apart from such

types which in all other biological aspects are identical with the mucus producers. Take for instance the organism of ropy bread, *Bac. panis viscosi*, which, on account of its mucus production, is regarded as a distinct species though in other respects it is identical with the aerobic soil bacilli. Or, take the cause of the so-called "frogs spawn" in sugar juices, which at present is known to microbiologists under a variety of names and as a quite distinct species, though in all respects, except for an excessive production of mucus, it is identical with the classical and widely distributed acidifier of milk and of other sugar-containing liquids. Mucus is produced by these types only under certain cultural conditions and is not specific to them. It is a phase in the metabolism of carbohydrates by micro-organisms exaggerated, when it becomes noticeable, by the occurrence of such conditions. You will appreciate, I think, the complications which may arise in cases where an epidemic of ropy bread, for instance, has to be traced to its source and remedies found for combating it, when one approaches the task, as has been done since the correct conception of mucus production was established, on the assumption that only such types of aerobic soil bacilli which produce mucus on ordinary culture media can be regarded with suspicion. In many cases such media would be incapable of inducing mucus formation even in the case of the most active strains.

No more striking example, perhaps, could be given of the practical importance of acquiring a detailed knowledge of the chemical products of metabolisms of micro-organisms than that of the rise to industrial fame of the bacillus now used on a very large scale indeed for the preparation of organic solvents. Before 1916 this organism, and the group to which it belongs, was well known to most microbiologists, so much so in fact that it was available in the various culture collections then existing, not as an industrially important type, but as a gas-producing anaerobe resembling, in many ways, the disease-producing members of the butyric acid bacteria. It required a chemist, and incidentally a world war, to demonstrate that this organism possessed properties which were of great industrial importance.

A detailed examination of its metabolism revealed that it produced, in addition to butyric acid, two valuable solvents in sufficient quantities to make it possible to recover them industrially. The demand for these solvents during the war was immense, and even to-day they are sufficiently great to justify the use of the organism in question for the conversion of thousands of tons of cereals every year into butyl alcohol and acetone.

The third example which I have chosen refers to a subject which has been overlooked practically by all microbiologists rather than monopolised by one section and left unconsidered by others. I am referring to the biophysical aspect of microbiology. In this field very important problems are awaiting solution, problems not only of great theoretical but many of practical importance. Take the study of the behaviour of micro-organisms under the influence of high-frequency sound waves for example, a study which has recently been commenced by Newton Harvey in America. It would appear from his preliminary investigations that the organisms which he tested were unable to withstand high-frequency sound waves and were destroyed. Is there not contained in these observations a clue to a new and important method for the destruction of micro-organisms other than by the old and extremely crude methods of heat application and antiseptics?

In all humility I have ventured to dabble with an analogous problem myself, and

I can say that my preliminary experiments have borne out my anticipations that a deeper understanding of microbiological biophysics will open up new and important fields of application in various directions.

Let me recall in this connection also the important question of the study of oxidation-reductions which nowhere can be explored more conveniently than among micro-organisms. Here again is a problem which may one day be of the greatest practical importance in the control of the growth and expansion of micro-organisms.

Another interesting biophysical problem is that of the production of light by micro-organisms. Certain bacteria, as you recollect, produce a bright yellowish green light, the wave-length of which is limited to a narrow band in the visible range. The efficiency of this conversion of energy into light by the phosphorescent bacteria is surprisingly high; it exceeds 80 per cent. according to Newton Harvey. Surely, a better understanding of this reaction could not fail to assist us in planning how we have to set to work to increase our efficiency in light production.

You probably consider that I owe you an explanation for having referred to the present method of sterilisation by heat as crude. I think I can do so by referring to the present unsatisfactory position of the trade in fresh milk. I dare say that you have followed the discussion which has taken place recently both in the Houses of Parliament and in the daily press on the subject of supplying cleaner milk to the public. You may have noticed also that it has been claimed that there exists to-day only one way of ensuring the removal of non-spore-forming micro-organisms from milk, and that is by boiling it. But boiling, as Prof. Stenhouse Williams has very rightly observed, is not a satisfactory method, since it alters both the taste and several of the physical and physiological properties of the milk. Something has evidently got to be found to replace boiling in the preparation of hygienic milk and, as I have ventured to suggest, I think it is in the direction of a control of those physical properties which govern the growth of micro-organisms that this "something" has to be looked for.

Having pointed out the shortcomings of an excessive specialisation it is only fair that I should endeavour to convey to you what, in my opinion, should be put in its place. In attempting to do so let me recall that in all branches of microbiology we are dealing with living beings which are subject to the restrictions imposed upon all life by its environment and its specificity and dominated by the two greatest forces of all life, growth and expansion. In all branches of microbiology it must be indispensable, therefore, to take account of the restraining influence of environment and of specificity as well as of the forces of growth and expansion. In other words it is essential in my view for all microbiologists to possess a sound knowledge of general biological principles, as well as of biochemistry and biophysics. Having acquired such knowledge it will be necessary for the microbiologist of the future to specialise, for it goes without saying that nobody can acquire expert knowledge in the whole field of microbiology. But I think that a wide outlook such as that suggested could not fail to create a greater bond of interest between the specialists than that which prevails to-day.

I cannot help thinking also that it was a feeling akin to this which prompted the recent Paris Microbiological Congress to appoint an international committee for the elaboration of a new and better co-ordinated microbiological nomenclature. The organisation of the work of this committee has been left in the hands of a number of national committees under the leadership of an Anglo-American secretariat, and

care has been taken to ensure that all branches of microbiology are represented on the various national committees.

A broad outlook and a more comprehensive knowledge of the various branches of microbiology on the lines I have indicated is perhaps nowhere more essential than in a section of microbiological study which has come into some prominence during recent years. I am referring to what is known as industrial microbiology. You will agree, I think, that this must be so, when you consider that, given the necessary amount of moisture, practically all organic, and many inorganic substances can serve as food substances for micro-organisms, and that through the action of such organisms these substances will be altered—favourably or unfavourably—and perhaps even destroyed.

A multitude of raw materials and merchandise is liable to become infected, altered and damaged during manufacture, transport and storage. Cotton and all other textile fibres and fabrics, including wool and silk, are subject to such damage which involves industrial undertakings in losses amounting to millions of pounds annually. In the manufacture and storage of sugar, of grain, of flour and of starch, similar conditions prevail. Timber and wood products, such as pulp and paper, are equally exposed, as are meat and fruit, paints, waxes, rubber, and perhaps even stone. Many of the problems associated with the study of the micro-organisms taking part in the destruction of these various substances could not possibly be dealt with on purely biological lines, but must require an appreciation of the biochemical and presumably of the biophysical characteristics of the organisms. And of course such appreciation becomes increasingly important in cases where micro-organisms are required as agents or catalysts for the preparation of required substances—not necessarily of wine or beer, but of silage, coffee and cacao, tobacco, organic acids, nitrogen compounds and of many others.

I think it is lack of comprehensive training and of knowledge rather than scarcity of problems which up to now has kept back developments in industrial microbiology.

With other workers in this field, in this country, in America and on the Continent I have tried for some years now to draw attention to the industrial application of microbiology, and I think that the feeling I have expressed here as to the advisability of such applications is now being increasingly felt. At any rate I see an indication of this in the attitude of scientific journals in America and on the Continent which for some years past have given increased space to subjects dealing with industrial microbiology. It is interesting to note also that in Germany a society was formed a few years ago for the specific purpose of furthering the investigation of industrial problems of a microbiological nature. But there, as well as in the United States and in this country, financial difficulties have hitherto prevented the most important step being taken, that of establishing an institute devoted primarily to the pursuit of the study of industrial microbiology. You may recollect that the question of establishing such an institute was debated at considerable length in 1923, at a joint meeting of the Biochemical Society and the Society of Chemical Industry, under the chairmanship of Mr Chaston Chapman, who has long been a spokesman for industrial microbiology. I think I am correct in stating that the overwhelming majority of those present at that meeting were in favour of the opening of such an institute. Unfortunately, however, fair words and pious wishes are not sufficient foundation on which to build an institute for the study of general and industrial microbiology, and it must be the duty of those, therefore, who are anxious to see this materialise to strain their efforts

to the utmost to convince the powers that be of the practical importance of industrial microbiology.

I am going to suggest that like the "Bund technische Bakteriologen" in Germany so the Association of Economic Biologists ought to make itself a spokesman for this cause in this country. In any case my chief purpose in addressing you to-day was to place this suggestion before you in the hope that you may be able to see eye to eye with me—at least so far as to sanction the opening of space in the *Annals of Applied Biology* for a section dealing with industrial microbiology. If you do, you will not be the first to take this bold plunge, for, as I have already indicated, both the *Biological Abstracts* in America and the *Centralblatt für Bakteriologie* in Germany are now devoting increasing space to this subject.

II. A GENERAL REVIEW OF THE MICROBIOLOGY OF CELLULOSE AND ITS ASSOCIATED COMPOUNDS.

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The microbiology of cellulose and its associated compounds, in particular the hemicelluloses, is a large and important subject, and yet the contributions to its study before, say, twenty years ago could almost be counted on the fingers of both hands, and such results as were obtained were disjointed. The activities of workers have increased enormously in the last few years in almost all aspects of Cellulose Microbiology, and there is also noticeably more co-ordination.

In Nature we see the microbiological destruction of cellulosic materials on the grand scale. Every year thousands of millions of tons of vegetable material are broken down by the microflora of the soil with an efficiency which, as a general rule, is unrivalled in the laboratory. The rate of elimination of vegetable debris in the soil depends of course on the conditions prevailing, being most rapid in a well-aerated arable soil, where the percentage of organic matter has been found to revert to a constant low figure in spite of the annual reintroduction of further dead vegetation.

Of the organisms responsible for this destruction in the soil, it may safely be said that all types, morphologically and systematologically speaking, participate; sometimes one group preponderating, sometimes another, according to the conditions—moisture, temperature, pH, etc.

The soil microflora of the hemicelluloses would appear to be as numerous as that of cellulose. The fungi are undoubtedly very active in the decomposition of these compounds, and from one's observations in the laboratory, employing a medium rich in xylan, it would appear that there are also a number of species of bacteria which give indications of being unsuited to a diet deprived of hemicelluloses.

The Actinomycetes probably play a much greater part in soil microbiology than is generally realised. These organisms are at times extremely numerous and active, though the rate at which they are able to break down cellulose is considered by some to be such that they cannot rank as the most important organisms in this respect. But one may safely say that between a third and a half of the soil species commonly isolated are capable of decomposing pure cellulose under laboratory conditions, and

that their action on hemicelluloses is probably at least as important. A particular feature of many of the Actinomycetes is their omnivoraciousness. It is quite common to find a species which, while disintegrating cellulose with comparative rapidity, will also hydrolyse starch readily, and is equally at home in destroying sugars, peptonising milk, decomposing gums and pectin, proteolysing gelatine and so on. Now the cellulose-decomposing bacterium is not like that as a general rule, it is restricted almost entirely to cellulose as its source of carbohydrate and it will sometimes not tolerate the *presence*, even, of other carbohydrates. In fact, as a general rule, while the cellulose-decomposing Actinomycete (and fungus also) is a *gourmand*, the bacterium is a *gourmet*. The rôle of the true bacteria will be referred to below.

Reverting to the soil, the question of the decomposition of cellulosic material is now receiving much more adequate attention, principally of course under the aegis of agricultural institutions, such as Rothamsted. It is not my object to deal with this aspect in detail, but let me just refer as an example to the relationship existing between the disposal of cellulose and the amount of nitrogen in the soil. If there is not symbiosis in the strict sense (if there is such a thing as symbiosis in the strict sense) it is a balanced state of interdependence which, in the natural processes of soil microbiology, is of unrivalled importance. There is, for example, a distinct relationship between the destruction of cellulose and hemicelluloses and denitrification. As Jensen points out, when manure is added to the soil large quantities of energy material are introduced, so that the micro-organisms develop abundantly, especially if straw is present. This means that a considerable amount of nitrogen is locked up in protoplasm and therefore nitrification is retarded until the numbers of micro-organisms have decreased again. The rise in nitrate production which sets in at this period corresponds to the decomposition of the dead bacteria. Our more accurate knowledge of the decomposition of plant materials in the manure heap dates really from the study of artificial farmyard manure. It is now a commonplace that, given the presence of the necessary micro-organisms, straw can readily be converted into a typical well-rotted manure, as good as the natural article, if moisture and nitrogenous food are added; the addition of chalk, as a neutralising agent, being also favourable. The organisms necessary to effect the change, which consists of a partial breakdown of the cellulose and hemicelluloses, do not have to be added because they are normally present on the straw. A somewhat higher temperature than that of the surrounding atmosphere has a beneficial effect, but this does not need to be artificially induced, as the heap will show a rise in temperature of its own accord if oxygen has free access to the heap.

The rise in temperature which occurs in the manure heap, and indeed, in the decomposition of all vegetable debris under suitable conditions was at one time attributed solely to purely chemical processes. Now, it is generally conceded that this heating is due to microbiological oxidation processes—or at any rate in its early phases. The microbiology of farmyard manure decomposition in the soil is now receiving a fairer measure of attention particularly from Jensen, who has been working at Rothamsted and whose papers on the subject are appearing in the *Journal of Agricultural Science*.

Here one should refer to the work of Prof. Miehe on the spontaneous heating of hay, which he has carried on for a number of years. The principal points of his recent monograph, *Über die Selbsterhitzung des Heues* may be summarised as follows:

Properly sterilised hay does not heat. When imperfectly sterilised hay heats, it is

invariably possible to show that bacteria are present. Air-dry hay does not heat unless the relative humidity of the surrounding air is at least 87 per cent. Antiseptics wholly suppress the power of heating. Pure cultures of bacteria in sterile hay cause heating, and although they may raise the temperature through only a relatively short interval, this may be very effective, as the thermophilic bacteria may raise the temperature beyond the limit (65° C.) at which the most thermophilic fungi can survive.

Miehe concludes that the rise of temperature up to 75° C. can be sufficiently explained by the respiratory action of fungi and bacteria, there being no arguments to favour the theory that heat production is due solely, or even in any appreciable degree, to the activity of plant enzymes. When a temperature of over 75° C. occurs—and one knows that sometimes spontaneous ignition occurs—the heat liberated must arise solely from chemical reactions, though it is unknown at exactly what temperatures these reactions start.

One has a practical application of the natural self-heating of plant tissues in the fermentation of tobacco. After drying, the leaves are stacked in heaps, and if the moisture content is sufficient, the temperature rises in the course of two or three days to anything between 48° and 70° C. Too high a temperature must of course be avoided, and this is done by rebuilding the stack every few days. The changes brought about involve not only the hemicelluloses of the leaves, but also the starch, glucosides, fats and proteins. There is still a considerable school which attributes the tobacco fermentation solely to plant enzymes, and there are others who attribute it to the presence of inorganic catalysts, such as iron, which are considered to accelerate not only the oxidation of the carbohydrates, but also the saponification of the fats and glucosides and the proteolysis of the proteins. The weight of evidence however, supported as it is by the strong analogies of the self-heating of hay, seems rather in favour of a microbiological explanation.

In the fermentation of the cacao and the coffee bean we have further instances of spontaneous heating, which are variously attributed to micro-organisms and to plant enzymes. The extent, however, to which the cellulosic materials are involved has hardly been investigated.

The conversion of green fodder into silage is another process in which the cellulosic materials are to a greater or less extent altered and the mass undergoes change with the spontaneous generation of heat. The conditions are different from those in the foregoing cases, however, in that access of oxygen is restricted and the temperature of the mass, which is dependent on this access of oxygen, is not allowed to go too high. The fermentation takes place, in fact, under water-logged conditions, and is complete before the cellulose and hemicelluloses have undergone more than a partial destruction. In fact it is largely on the content of these bodies that the nutritive value of the product depends.

Opinions on the responsibility for the process of ensilage have oscillated between micro-organisms and plant enzymes, but the weight of evidence in the last few years comes down heavily on the side of the micro-organisms.

So far, one has been considering cellulose in the state in which it exists most commonly in nature, in intimate association with other compounds. It is necessary to consider the substance in a more restricted sense, since the laboratory worker must, of necessity, do much of his work on the pure substance, if he hopes correctly

to interpret the phenomena he observes in more complex systems where more than one substance and a greater number of factors are present.

Cellulose as it exists in the cotton hair, and the bast fibre of the flax plant, and so on, is in an almost pure state, and it is with cellulose in this state that the micro-biologist interested in the micro-organisms decomposing cellulose has to work in the laboratory. It is in cellulose in this state, also, that the textile industries are interested. From the moment of the formation of the cotton hair in the boll of the plant to the time of its appearance in a manufactured fabric—and after, the cellulose of the cotton hair is at the mercy of a great variety of micro-organisms, which do damage costing millions of pounds a year. It has been found that directly the moisture content reaches 8 per cent. the growth of some of the cellulose-destroying organisms which are invariably present on the cotton hair may start. As the normal moisture content is not 1 per cent. less than this, the margin of safety is small. Until the cotton bales reach the mills, the only effective measures that can be taken are adequate ventilation and the elimination of moisture. In the mill the size added to the yarn is an additional complication. It is usually a good nutrient medium for micro-organisms and so increases the susceptibility of the fabric to damage during storage and transport. This is usually combated in practice by the addition to the size of antiseptics, but this is not as simple as it sounds. The ideal antiseptic for the purpose has to possess the following virtues: it must be cheap, have no action on the cellulose, be colourless at all reactions, and not affect dyeing or finishing processes, must be odourless, non-volatile, sufficiently soluble to be uniformly distributed in the size, must be unaffected by heat, by the metal of the machines with which it comes in contact, or by other ingredients of the size or finishing materials. Furthermore, it has to have the unique property of being highly toxic to micro-organisms, and at the same time being perfectly harmless to the textile worker should he get it on his body or in his mouth.

A further difficulty arises in that most antiseptics show marked specificity in their toxicity. Some organisms are readily killed while others are unaffected by the same antiseptic.

When you have found your ideal antiseptic, there are still some objections to its employment. The introduction into the size of a toxic substance conforming to the conditions I have enumerated may suffice in most cases in the textile industry, where the subsequent removal of the antiseptic in laundering is not of great moment, but there are circumstances in which this is a distinct disadvantage. In the case of tentage, or canvas equipment or other material which is subject to frequent saturation with moisture, and hence to the removal of any antiseptic incorporated into the size, such a mode of protection is obviously insufficient. The demand for a suitable treatment to meet such cases as these is sufficient to warrant the further attention of workers in this field. Two possibilities occur to the mind. It might be possible actually to impregnate the cellulose of the hair or fibre, not just to coat it. The physical properties of the fibre must, however, be in no way altered, and the antiseptic *must* be to some extent soluble otherwise it cannot function. The alternative is more radical, but is also the ideal. This is the possibility of an alteration in the actual cellulose of the fibre so that it is no longer a suitable source of food for the micro-organisms. Any such alteration must, of course, be slight, and the valuable physical properties of the fibre must be unimpaired. Such a suggestion may sound fantastic, but in the laboratory such a method has met with a considerable measure of success.

Systematic scientific work on the destruction of fibres and fabrics by micro-organisms has really been taken in hand only during the last ten years, so that we have still a long way to go. An early observation still awaiting confirmation or contradiction is the difference of rate of destruction of cottons of different origin recorded several years ago by Dr Thaysen and myself in laboratory experiments. Further evidence for or against this belief may be forthcoming shortly when it will be possible to report on the first part of investigations for the Fabrics Research Committee of the D.S.I.R. into the behaviour of a number of fabrics when exposed to microbiological destruction under various conditions at a number of stations in different parts of the world. In passing I might just mention here that the microscopical detection of damage in cellulose hairs and fibres is greatly facilitated and often only made possible by the swelling test of Fleming and Thaysen, and more recently by the congo red test of Bright. By these tests, not only is damage to the fibre detectable at a much earlier stage than would otherwise be possible, but also, microbiological damage can be differentiated from non-microbiological, such as chemical, heat or light damage.

It is no doubt the increased realisation of the economic importance of a study of the microbiological breakdown of cellulose and its associated compounds in some of the fields I have enumerated that has led to the very marked increase during the last ten years of laboratory study of the micro-organisms concerned in this breakdown.

As I have mentioned, the organisms responsible for cellulose decomposition cover a very wide range, and the study of some of them in the laboratory presents special problems. The cellulose-destroying fungi present no special difficulty, as they can be readily isolated in pure culture and their activities can therefore be studied the more readily. It is the same with the cellulose-destroying Actinomycetes. It is the bacteria which are the most difficult to deal with. Many of them refuse consistently to develop on solid media, and have not, therefore, been isolated in pure culture. It is true, some have said "Why bother?" But this attitude, besides being shocking and heretical, cannot be maintained, because so often one has found that the reasonable scientific investigation of a cellulose-destroying species has become impossible in a mixed culture, through the presence of one or more infections. Others, despairing in their efforts to isolate the required cellulose decomposer in pure culture have affirmed the existence of a symbiotic relationship between the cellulose decomposer and one or more of the "infecting" types, without which, it is alleged, the cellulose decomposer *cannot* exist.

In *some* other cases in which species of alleged cellulose decomposers have been isolated on solid media, it has had to be admitted that these cultures have, after isolation, lost their power of destroying cellulose. In at least some of such cases, what has finally been obtained has been a pure culture of the infecting organism.

Nevertheless, by the application of a variety of different methods, a certain number of cellulose-destroying organisms have been isolated either in pure culture or in a sufficient state of purity for their main physiological reactions to be studied. The first of such types to be recognised were, of course, Omelianski's *Bac. methanigenes* and *Bac. fossicularum*, readily obtainable from mud. An interesting anaerobic type isolated by Mme Khouvine from human faeces is *Bac. cellulosae dissolvens*. The organism will not develop satisfactorily unless there is present in the medium an extract of faecal matter. In passing, I should say that I have omitted to discuss the

question of the intestinal decomposition of cellulose. As in so many other spheres, the breakdown of cellulose in the alimentary tract was at one time considered to be due solely to the activity of enzymes secreted by the alimentary canal itself, but that view can now be definitely abandoned. Nevertheless, very little is known of the flora responsible for the cellulose decomposition, and one can only say that it appears to embrace a variety of types; spore-forming rods, Actinomycetes, cocci and even fungi.

Turning to aerobic forms, undoubtedly one of the most interesting types is Hutchinson and Clayton's *Spirochaeta cytophaga*, which is probably identical with the organism described some twenty-eight years ago by van Iterson as *Bact. ferrugineum*. This organism, so active in the soil, and indeed apparently in all places where the natural decomposition of cellulose is proceeding under aerobic conditions, is of particular interest because it appears to exist under two forms, a slightly curved rod and a coccoid. For this reason, the purity of the organism was suspect, but those of us who have kept cultures of this organism for many years now have little misgiving on the subject. In fact, some of us, in our heart of hearts, until recently felt that this is the one culture of a cellulose-decomposing organism the purity of which rivals that of Caesar's wife. Winogradski, however, considers that the organism is not pure. He describes a number of species allied to *Spirochaeta cytophaga*, which he has secured in a state of purity sufficient to enable him to study their reactions. These organisms are not really Spirochaetes, of course, and Winogradski realises the misnomer, and puts them into a genus *Cytophaga*.

Kellermann, McBeth and their collaborators in America have isolated a number of aerobic types which they claim decompose cellulose and which are of particular interest in that a solid agar medium containing precipitated cellulose was employed for their isolation. The growth of the organisms on this medium is accompanied by the production of clear zones round the colonies, where the cellulose has been resolved by the bacteria. This appearance is quite striking in some cases, and is due to a disappearance of the cellulose and not, as has been suggested by critics who have not seen the phenomenon themselves, simply to the elimination of calcium carbonate which is present in the medium. Kellermann and his collaborators found that some of their cultures of cellulose-decomposing bacteria obtained in this way soon lost their power of decomposing cellulose. Some critics maintain that an examination of the clear zones shows them to be teeming with bacteria, which they contend are the real cellulose decomposers, the large colony in the centre of the cleared zone being an infection. It is maintained that the reason why cellulose decomposition and zone formation fail to materialise after two or three sub-cultures is that the true cellulose decomposers, present in the zones, die off and one is finally left with a pure culture of the infection, not of the cellulose decomposer. One can only say that the several species one has oneself isolated which do produce this zonation round the colonies and which may or may not be identical with Kellermann's species, certainly have every appearance of being pure and they do decompose cellulose. Skinner and Kalnins have also obtained similar results.

Quite a number of other types of cellulose decomposers have been described during the last few years, for example by Winogradski, Dubos, Kalnins, and before leaving them I should refer to the thermophilic cellulose decomposers. These constitute a group which is undoubtedly not only of great interest, but probably also of great economic importance. Several types have been described, and some are remarkable

for their efficiency and their rapidity of action. One has several times come across species, not in absolutely pure culture, which are capable of reducing pure filter paper cellulose to a yellow incoherent mass in less than 48 hours at a temperature of 60–65° C. It is more than likely that an organism of this group was responsible for an interesting case which came to one's notice of damage to a fire-hose pipe which was left in a saturated condition at a moderately high temperature, and which was completely rotted in the course of two or three days.

These thermophilic types are by no means rarities. They can be obtained without difficulty from almost any good garden soil or manure, and they no doubt take an active part in all those processes of decomposition of vegetable debris, some of which I have mentioned, where a heating of the mass takes place.

It will be realised, then, that the cellulose-decomposing bacteria cover a very wide range, not only morphologically, but also physiologically, and hence the products of their reactions show considerable variety.

This raises the subject of the industrial application of the microbiological decomposition of cellulosic materials. Natural gas, hydrogen and methane, have been successfully obtained for several years in one or two places by the fermentation of waste vegetation. It should be noted that one deals in such cases, not with one material, such as cellulose, or xylan, but with a mixture of materials of varying composition. The bacteria employed in such fermentations are also mixed, in fact the activity of the responsible organisms has in some cases been found to have been definitely increased by the presence of other types. On the other hand, the employment of mixed cultures in an industrial process is often open to grave objections, in that the difficulty of controlling your cultures, and hence your yield, is greatly increased. In the decomposition of plant material the ideal organism would be one which decomposed cellulose and lignocellulose with equal facility, as these are the substances most abundantly represented in vegetable tissue.

The same remarks apply to the much more important economic problem of the conversion of the cellulose and hemicelluloses of waste vegetation into alcohol for use as a fuel. There are two methods by which this conversion may be brought about. One method is to subject your waste vegetable material to a preliminary hydrolysis with acid, and then to ferment the resultant sugars. Thaysen and Galloway, who have made a detailed study of this method on a semi-technical scale, have met with a considerable measure of success.

The other method, undoubtedly more advantageous, consists of a direct fermentation of the plant material without any intermediate hydrolysis, but its practical industrial application is at present hindered by various difficulties. The complicated composition of most waste vegetable material is not the least of these, and suggests the necessity of employing perhaps three or more organisms simultaneously if anything like a complete conversion into ethyl alcohol is to be secured. This makes a proper control of the fermentation extremely difficult, and at present it is necessary to limit the bacterial production of alcohol to a fermentation of the cellulose, *or* the hemicelluloses, *or* the lignocellulose of the plant debris.

A number of successful fermentations of cellulose to yield alcohol on the small and semi-technical scale have been reported, both mesophilic and thermophilic organisms having been employed, and one believes the Distillers Co. have met with a considerable degree of success in some of their experiments, though results are not

published. Similarly an amount of work has been done on the direct fermentation of the hemicelluloses, in particular of xylan. From one's experience in the laboratory, there is an extensive flora which thrives on a nutrient medium rich in xylan, and in some cases gives distinct morphological indications of being adversely affected if deprived of this hemicellulose. On a xylan agar medium, one frequently notes the formation of clear zones round the colonies of xylan-destroying organisms, analogous to the zones which surround the cellulose-decomposing bacteria on cellulose agar. Werkman and his collaborators in the United States are studying this question of the direct fermentation of hemicelluloses. As with the fermentation of cellulose, the products consist of alcohol and organic acids.

I have said nothing about lignin. This is really of considerable importance because the great bulk of cellulose which is subject to microbiological breakdown is in the form of lignocellulose. We know that as a general rule lignin is more resistant to attack by micro-organisms than are cellulose and hemicelluloses. Nevertheless, its destruction by some of the higher wood-destroying fungi is manifest in the case of so-called "white rots," where the lignin is removed from the woody tissues of the wood, leaving white patches of cellulose. In the "brown rots," the reverse occurs, the cellulose being destroyed and the lignin left unattacked.

The proper study of the microbiological breakdown of lignin and the substances with which it is associated in nature has so far been greatly hindered by the obscurity of the chemistry of the substance and the inability to obtain it in a pure form. Laboratory experiments on the microbiological degradation of lignin have been carried out, but there is a strong suspicion that the violent chemical reactions which lignocellulose has undergone in order to obtain the lignin have modified its chemical structure to such an extent that one is no longer dealing with true lignin. The surmounting of this difficulty will hasten the proper investigation of the microbiological destruction of lignin and lignocellulose, and may not only lead to results of economic importance, but also assist us to understand more fully the processes involved in peat and coal formation, a subject of considerable theoretical importance.

The widely prevalent view on peat and coal formation, influenced by the observations of Fischer and Schrader in Germany, is largely taken from a wrong standpoint, the assumption of what occurs in the earlier stages of the process being based on an imperfect grasp of the limitations which it is clear to the microbiologist must exist under the conditions actually prevailing in the peat bog. Broadly speaking the Fischer and Schrader conception is of a gradual but complete elimination of the cellulose from the accumulating plant debris, this elimination taking place through the action of micro-organisms down to a considerable depth, and eventually leaving the lignin, which they consider is the only essential from which the peat and coal are formed. They regard fungi as being mainly responsible, in spite of the fact that the water-logged condition of the peat bog is not a suitable one for the active development of these organisms. They quote as evidence the analysis of a wood in which fungal activity had eliminated the cellulose and left the lignin, regardless of the fact that equally good figures are available of other fungus-attacked wood in which the lignin has been eliminated and the cellulose left behind.

It must be conceded that the bacterial decay of pure cellulose does not, as far as experience at present shows, lead to the formation of humic substances (though even here Winogradski disagrees) and it is even granted that in more cases than not, the

wood-destroying fungi eliminate the cellulose and not the lignin, but in considering the problem one must bear in mind the actual natural conditions under which the conversion to peat and coal takes place, namely under water-logged conditions, in the more or less complete absence of oxygen. It is, in fact, under conditions in many ways resembling those prevailing in the preparation of silage, and the comparison is not without value. In the silo, microbiological activity ceases long before there is anything approaching a complete decomposition of the cellulose, or of much more readily decomposable constituents. Is it likely, therefore, that in the peat bog—where conditions of temperature and the nature of the material to be decomposed are certainly less favourable—the cellulose will be completely eliminated?

We can do better, however, than argue solely by analogy. There is now ample experimental evidence to show that peat still has much and in some cases the bulk, of its cellulose left undestroyed at depths at which the organisms which could accomplish the destruction, the anaerobic cellulose decomposers, have ceased to exist. They have not, in fact, been found at a greater depth than about four feet.

The elimination of cellulose in the formation of peat and coal must therefore be performed by some agency other than micro-organisms.

REVIEW

A Textbook of Agricultural Entomology. By Kenneth M. Smith. Pp. xiii + 285, with frontispiece and 79 illustrations. Cambridge: University Press, 1931. Price 12*s.* 6*d.* net.

The standard work on English agricultural entomology for many years has been Curtis' *Farm Insects* (1860). This book has maintained a long ascendancy on account of its detailed treatment of the subject and the excellence of its illustrations. It is now obviously very much out of date and the need for a modern treatise is generally recognised. Dr Kenneth Smith is evidently well aware of this requirement since he states, in the introduction to his book, that he had in mind the preparation of a volume comparable in size with that of Curtis. This good intention, however, had to be dismissed owing, he says, to the high costs of production that such a work would entail. Since Curtis' time several elementary books dealing with agricultural pests have appeared. For the most part the treatment of the subject in such manuals has been very brief and, more often than not, agricultural and fruit pests have been included in a single volume. In Dr Kenneth Smith's book we have a work devoted exclusively to the insect pests of farm crops and farm animals. By thus limiting its scope, he has been able to give more detailed consideration to his subject than will be found in preceding manuals of a similar kind.

A feature which catches the eye upon opening this book is the absence of any introductory account of the elements of entomology. This omission is justified for the reason that unless there is space to treat the subject adequately (and in the present book there is not) it is far better left out. There are three introductory chapters. Chapter I describes briefly the organisation of agricultural entomology in England and Wales, with special reference to the Phytopathological Service and to the Imperial Institute of Entomology. At the end of this chapter there is a list of books and journals, of a more or less general character, bearing upon insects and control methods. Chapter II outlines the different types of control measures and Chapter III discusses weather conditions in relation to epidemics of insect pests. These three chapters are very short, but they outline, at any rate, essentials. There follows eleven chapters dealing with specific farm pests: these latter are grouped according to the orders to which they belong and are consequently not arranged upon a crop basis. The method adopted is to give a concise description of each pest in the adult stage, followed by some account of its immature phases. The life history, host plants, injuries and symptoms of attack, distribution, control measures and natural enemies follow in the order mentioned. At the end of each chapter is a guide to the literature dealing with pests mentioned. These bibliographies being well chosen and up to date, will provide the enthusiastic searcher for knowledge with all the main sources of original information. The last chapter (XIV) is a special feature in that it discusses insects and their relation to the transmission of virus diseases of plants, a subject upon which the author is a recognised authority. At the end of the book there are two very useful appendixes. Appendix I is a crop list giving tabulated symptoms of attack and the names of the insects responsible for specific injuries. Appendix II lists the commoner farm weeds that serve as alternative hosts to certain insect pests, which are also enumerated. The indexing of the book is very efficiently done, there being an index of authors, one of parasites and predators and also a general subject index.

Viewed as a whole the general impression gained of this book is that it is very well arranged and very well produced. At a cost of 12*s.* 6*d.* it is distinctly cheap. It is clearly written and, owing to its compressed style, it contains a very considerable amount of information in a relatively small compass. The outlook of the author is both practical and scientific and this feature is reflected to advantage throughout.

He has explored recent literature very conscientiously, besides adding the results of his own independent observations. The book, therefore, is up to date and it is also pleasing to note an entire absence of perennial over-worked illustrations, dug up from the archives of antiquity, that have so often done service in publications bearing upon agricultural entomology. The illustrations are clear and exact: some are original and the remainder are well selected from recognised modern sources. Bearing in mind that the scope of the book is necessarily limited by its size, we have no quarrel with its author on the score of omissions. Here and there there are small points wherein differences of opinion will arise, but actual errors seem to be remarkably few. We have been able to find but few misspellings or misprints and are glad to acknowledge this fact. We think, however, that if a new edition be called for, one or two very minor emendations might be taken into account. Fig. 38, for example, really refers to *Cephus cinctus*, and not to the closely allied *C. pygmaeus*. On p. 212 we raise the question as to whether the wild host of the wheat bulb fly has been definitely proved to be couch grass. On p. 101 the characters given for separating the larvae of different chafer beetles include no reference to those afforded by the anal segment. The latter region, it may be added, affords easily recognisable features separating *Melolontha* from *Phyllopertha*, for example, that can be detected with a pocket lens. Minor points of this nature really detract so little from the value of the book that their mention, in a general review of this character may seem to savour of being captious.

We congratulate the author on the production of a book that is of both scientific and practical utility. As a general guide to farm pests it should prove helpful to a considerable circle of readers whether they be students, teachers, or agricultural officers.

A word or two by way of suggestion may be opportune here. There seems to be room for two other volumes uniform with the present one. An up-to-date handy treatise on fruit and kindred horticultural pests would be a useful addition to English entomological literature. Also, a manual dealing with insects affecting stored products and manufactured goods is very much of a desideratum at the present time.

A. D. IMMS.

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THE FORMATION OF INTRACELLULAR INCLUSIONS IN SOLANACEOUS HOSTS INFECTED WITH AUCUBA MOSAIC OF TOMATO[1]

BY F. M. L. SHEFFIELD, PH.D., F.L.S.

(*Department of Mycology, Rothamsted Experimental Station, Harpenden.*)

(With Plates XXV–XXXIII.)

CONTENTS.

INTRODUCTION.

MANY virus diseases of plants and animals produce abnormal inclusion bodies in the cells of the host. These were first observed in plants by Iwanowski (10), who described the inclusions which appear when tobacco is infected with mosaic disease. His observations have since been confirmed by a number of workers (2, 3, 4, 6 and 19), but the phenomena observed have been interpreted in varying ways. Similar cytological effects are caused by this disease in other hosts (8), and intracellular inclusions are produced in the host plant by many other virus diseases such as curly top disease of sugar beet (18), dahlia mosaic (7) and virus diseases of potato (8, 25). They are formed also in the cells of diseased monocotyledonous plants such as sugar cane infected with mosaic

[1] A grant in aid of publication has been received for this paper.

disease (2, 14), in wheat with rosette disease or with a mosaic-like leaf mottling (16) and in Hippeastrum mosaic (9, 13).

When aucuba mosaic of tomato is inoculated into *Solanum nodiflorum* large conspicuous protein bodies are produced in many of the cells of the host plant (24). A short note as to their mode of origin has been given by the writer and J. Henderson Smith (21). It is proposed now to give a fuller description of the development of these intracellular inclusions and also to compare the reaction of other hosts to this virus.

Material and methods.

The main part of the paper concerns *Solanum nodiflorum* infected with aucuba mosaic of tomato (22). *S. nodiflorum* is an exotic species closely related to the British forms *S. nigrum* and *S. dulcamara*. It is of no economic importance, but has been selected for intensive study as it shows some very clean cut cytological effects. The action of the disease was studied also in the cells of several other Solanaceous hosts, viz. *S. nigrum* (black nightshade), *S. lycopersicum* (tomato), *Nicotiana tabacum* (tobacco), and *Hyoscyamus niger* (henbane). The plants were grown in insect-proof glasshouses. Seedlings were inoculated with the virus by leaf mutilation.

Fixing and staining methods.

An attempt was first made to study the cell contents of leaves of *S. nodiflorum* from microtome sections of fixed material. A large number of fixatives were tried, including chrome-acetic mixtures, Flemming's fluids and several of their modifications, Bouin and Allen's modified Bouin, Zenker and Helly's modification, Worcester, Gilson, etc., as well as mitochondrial fixatives such as Altmann, Benda, Regaud, Champy and the osmic impregnation methods described by Bowen (1). Very little success was attained by any of these methods. It is sometimes possible to fix fully formed inclusion bodies without their being greatly distorted, but in the earlier stages of development of the disease the contraction of the cytoplasm due to poor fixation obscures the effects of the virus. The cells of the palisade tissue, epidermis and especially of the hairs of the leaves of this species are all very vacuolate; moreover, the cell walls are rather thick and the epidermis is cuticularised. Also the hairs tend to hold the air and prevent the fixing agent from reaching the epidermis. Consequently if a fixative is sufficiently strong to penetrate the tissues rapidly, it is impossible to avoid plasmolysis within the cells. Weaker fixatives are unable to penetrate the protective layers before

the cell contents show signs of death. To assist penetration the leaves were cut up into small portions and sometimes the epidermis was stripped from one surface. If before fixing in one of the weaker fluids the tissues are immersed for a short time in absolute alcohol, chloroform or Carnoy's fluid, slightly improved fixation is sometimes obtained. These methods have not yet been extended to the other hosts used where penetration would probably be easier.

Some difficulty was also experienced in finding a differential stain for infected material, both nuclear and cytoplasmic stains being taken up by the inclusion bodies. Feulgen's fuchsin sulphurous acid stain, the only specific stain for chromatin known, was used in combination with a dye such as Orange G, light green, methylene or aniline blue as a counterstain. The inclusion bodies then took on a somewhat deeper colour than the cytoplasm.

As the study of such fixed and stained material gave no very useful data it was abandoned for a time. Such results as were obtained served only to confirm the observation made on living material.

Vital methods.

Hand sections mounted in water or sugar solution, sometimes coloured with a vital stain, were used, observations being made only on uncut cells. Unfortunately, such cells remain in their living condition for only a few minutes. The epidermis can be preserved in the living state for a longer period if it is stripped from the leaf and mounted separately. It was found that a study could be made very simply of the cells of the hairs which cover the leaf surfaces of many Solanaceous plants as the difficulties of technique in the handling of living material are minimised. Also this study was the most productive of results, since intracellular inclusions are formed in these tissues with greater regularity than elsewhere in the plant and usually the cells are comparatively free from other inclusions. The hairs of *S. nodiflorum* are quite stiff, and a row of them project from the leaf margin. If a strip be cut from the edge of the leaf and mounted in water, a single living cell can be kept under observation for some hours. The thick wall which delays penetration by the fixing fluids, now prevents penetration by the mounting medium. In some of the other hosts, these cells are not quite so convenient to work with as the hairs are longer, softer and thinner walled, but always they are simpler to examine in the living condition than are any other tissues.

To study the development of inclusions formed as a result of infection with a virus, a leaf was detached from the plant at a suitable period after

inoculation. It was mounted in water on a microscope slide, care being taken to immerse the petiole. The hairs at the margin of the lamina were examined; if they appeared as in a healthy plant, the leaf was removed from the slide and the petiole was immersed in a nutrient solution. At intervals the leaf was remounted and re-examined. In this way it was kept alive for several days, and the whole process of development of intracellular inclusions was traced in the cells.

Attempts were made to stain the cells of the growing plant. A leaf lamina was removed and the cut petiole immediately plunged into a tube containing a solution of a vital stain such as dahlia violet, trypan blue, Janus green, neutral red, Bismarck brown, iodine green or methylene blue. All of these dyes were carried up in the water stream. The cell walls often became stained, also the contents of the glandular hairs, but no dye could be found to penetrate into any of the other cells.

If seedlings are inoculated with a mixture of crude virus juice and the dye, the plants become infected with the virus, but again only the cell walls are stained.

Photographic methods.

It was desirable to demonstrate many of the observations by photomicrographs. It was often impossible to take such photographs by the usual methods as, owing to the rapidity of movement within the cells, the plates of any ordinary photomicrographic apparatus could not be changed sufficiently quickly to take a series of photographs at frequent intervals. Resort was taken to the cinema camera, and the entire process of the formation of virus bodies in *S. nodiflorum* infected with aucuba mosaic was filmed. Many of the figures illustrating this paper are enlargements from single pictures of this cinema film. In some of them detail is ill-defined, not because the object is out of focus but because of its rapid movement. As the illuminant, oxy-acetylene gas with a thorium pastille was used; this illumination was not sufficiently intense to allow the ciné film to be taken at the normal speed of sixteen pictures per second. Actually the exposure given for each picture was twelve times as long as is usual, thus accounting for a lack of definition in moving objects when viewed on a single photograph of the series. However, the consequent speeding up when projected renders evident slow movement which is not discernible visually.

For all microscope work, both visual and photographic, transmitted light was used. If dark ground illumination be used, the thickness of the object causes much of the light to be lost by refraction from its

surface and only a very poor image of the cellular contents is obtainable. With transmitted light an appreciable amount of contrast can be obtained between the rather translucent object and the background by closing the iris diaphragm of the substage condenser.

Description.

Solanum nodiflorum and Solanum nigrum.

These two species are closely related and, apart from the obvious morphological differences, the only variation observed was a slightly greater permeability of the cell walls of *S. nigrum*. Otherwise it was impossible to distinguish the two forms histologically or cytologically. They will therefore be treated together.

The normal plant.

The leaf lamina consists of a single layer of closely packed palisade tissue, containing innumerable chloroplasts, towards the upper surface and about four layers of more loosely packed parenchymatous tissue below. The whole is covered by a cuticularised epidermis; many of the epidermal cells, especially those on the upper surface and at the leaf margin proliferate into hairs. Some of these are glandular, but the majority are of the protective type. Each of the latter is horn-shaped, curving round towards the leaf tip. All cell walls are very thick and the whole outer surface of the hair is minutely papillose.

Each hair consists of three or four vertically seriated cells, the lowermost is the largest, the others tapering gradually towards the apex. Each cell is a highly vacuolated structure (Figs. 1, 26). The wall is lined with a very thin layer of cytoplasm in which are embedded innumerable highly refractive particles, mitochondria and oil globules. Plastids occur in the hairs of very young leaves, but are not usually found in older hairs. The plasm flows continuously around the cell carrying these minute inclusions with it. Fine strands of plasm may flow across the central vacuole. Often these are thrown out from the peripheral layer. They may soon break and the torn ends flow again into the parietal plasm. Such strands often anastomose, but they may break apart again quite soon. Sometimes they lie in such close juxtaposition as to appear as a single thread, until on closer examination this is seen to consist of several strands in which the cytoplasm may be flowing in contrary directions. Occasionally the plasm seems to reverse its direction of flow: this may be due to two threads being superimposed, then one breaking away or being lost to view.

Each cell contains a nucleus which is carried passively about the cell by the streaming plasm, but its movement is never so rapid as that of the small inclusions and the plasm which carries them. Often it is spherical, but changes its shape as it is caught in the varying currents of the plasm. It may come to rest for a while against the cell wall when it usually assumes a lenticular form and the cytoplasm then flows around it. However, it is soon carried away again by the plasm. It may be rolled along against the wall or flung out towards the centre of the cell. Sometimes it is suspended in the vacuole by strands of flowing plasm which radiate from it towards the cell wall. Frequently anastomoses occur between threads, but when a large number of strands meet they are usually found to converge upon the nucleus. The cell contents may maintain such a state of equilibrium for a while, but ultimately some of the threads break away and the nucleus collapses against the cell wall or it is again caught up in a stream of flowing plasm. The degree of activity of the cell contents may vary with the temperature and illumination, but they are never actually at rest. Sometimes no movement is discernible by the eye, but even then it can be observed by magnifying the speed of motion through the medium of the cinema camera.

The diseased plant.

For a few days after inoculation no macro- or microscopic differences are observable in the host plant. However, about the fifth day, if growing conditions are good, there is a clearing of the green colour from the region of the veins, the first sign of chlorosis. At about the same time definite symptoms are seen within the hair cells. It is not known how long the virus takes to reach the individual cells, hence we have but little idea how much time elapses between inoculation of the individual cells and their reaction to the virus. The first effect of the virus to become obvious microscopically is an increasing activity of the cell contents. The cytoplasm becomes more conspicuous and may appear to increase in bulk. It flows with increasing rapidity around and about the cell. Thicker threads composed of many closely packed strands, in which the plasm may be flowing in contrary directions, as well as fine threads may appear across the vacuole. As in the healthy plant these are soon again absorbed into the peripheral plasm and fresh ones appear in new places. The nucleus continues to be buffeted by the flowing plasm, and it may be moved about the cell somewhat quicker than before.

The development of intracellular inclusions.

After some time a number of very small particles appear in the streaming cytoplasm (Fig. 2). These are of a faint yellowish colour, are angular and highly refractive. They are carried about the cell, their movement being comparatively rapid. More and more particles appear until they are quite innumerable. The speed at which they travel varies: if illumination or temperature is increased the rate of flow of the cytoplasm becomes greater with a corresponding increase in the speed of movement of the contained particles. Those in the parietal cytoplasm often tend to move more slowly than those in the strands crossing the vacuole. Often a particle halts for a while, but ultimately it is moved on again. Such a temporary cessation of movement occurs most frequently when it reaches the point of convergence of several strands of cytoplasm; at such a junction the particle may stay for a time before it is caught up in one of the diverging currents. Often particles are brought together in this way (Figs. 6 *a* and *b*), and they may fuse together either immediately or they may pass along a thread of plasm together for a while and fuse later. Sometimes one of these particles, after moving steadily along a cytoplasmic strand, will suddenly dart a short distance and then resume its former steady pace; possibly it has encountered a path recently traced through the colloidal plasm by a similar moving particle. In this way one particle may overtake another which is moving more slowly (Figs. 7 *a–e*) and they may unite. Sometimes particles come together for a while only to be caught in diverging currents and drawn apart again. However, fusions are comparatively frequent and the particles become fewer in number and larger in size (Fig. 8). Often a number accumulate about the nucleus, for, always if it is suspended in the cavity and sometimes even if apposed to the cell wall, it is the point of convergence for many of the cytoplasmic strands. It must not be regarded as due to any special property of the nucleus that the cytoplasm surrounding it is often studded with these particles, for they almost always accumulate if a number of strands converge on any one point for an appreciable time. Often particles lie against the nucleus for some hours, then by some new activity of the streaming cytoplasm they are whirled away to be again carried about the cell.

The streaming of the cytoplasm and the consequent movement of the particles is ceaseless. By aggregation and subsequent fusion the particles increase in size (Fig. 9). As they so increase their movement becomes somewhat slower (Figs. 10 *a* and *b*). There is no evidence at

any time that it is autonomous: the change in speed is probably partly a mechanical effect due to the increasing mass of the particles. Also the rate of cytoplasmic streaming appears to be gradually decreasing. By this aggregation the number of particles is gradually decreased until after a day or so a few large masses circulate slowly about the cell (Figs. 11–14). These masses are irregular in shape and consist obviously of innumerable loosely packed particles. They are plastic and change their shape as they are carried in the plasmic stream. A few small particles are still circulating freely, but almost all of them are gradually absorbed into the larger masses. The masses become more compact, but occasionally a fairly large portion will break away from the aggregation (Figs. 12, 22). This moves around the cell and ultimately it may again unite with the mass from which it broke away or it may join up with one of the other aggregations of particles.

A number of microchemical tests were made on the particles and on the masses which they later form at all stages of development in the living cell side by side with similar tests on the fully developed inclusion body. The latter have been fully described by Henderson Smith (23) and need not be dealt with in any great detail here. Except when using very strong reagents it is often difficult to be certain that penetration has occurred, but the general results obtained point definitely to a protein nature. Particles of all sizes, and the large aggregations are, like the completely formed inclusion body, insoluble in water, ethyl alcohol, xylol, acetone and chloroform. They are rapidly soluble in 10 per cent. KOH and in strong concentrated mineral acids. With osmic acid a brown coloration is usually obtained, but if material is fixed by the osmic impregnation methods of Bowen a blackening of particles and of the inclusion body occasionally results. However, no reaction was given with Sudan III or IV. Iodine appears to deepen the natural yellow colour of the particles and fully formed body; a similar result is obtained by the xanthoproteic reaction. A very definite protein reaction at all stages is given by Millon's test, the completed inclusion body and the larger aggregations giving a red coloration, whilst the smaller particles assume a somewhat paler tint of red.

The protein masses gradually unite together until practically all the material is contained in a single loosely packed, large and very irregularly shaped body (Figs. 15, 31), portions of which may break away only to rejoin later. The mass soon becomes much more compact, thereby considerably decreasing its volume; it loses its irregular outline, usually becoming roughly spherical. It happens occasionally that all the protein

material does not unite into one large mass, but two may be formed which round off independently (Figs. 21, *a–d*). They may fuse later, but usually result in the ultimate formation of two more or less spherical inclusions. The inclusion body, or bodies, are now completely formed. Small portions may still break away, and after a shorter or longer period rejoin the main body. A few very small particles may still be moving about the cell independently of the large inclusion body. Sometimes they apparently join it only to move away again, or they may be absorbed into its substance.

The distribution of these bodies throughout the host plant will be dealt with later, but in whatever tissues of *S. nodiflorum* these bodies have been observed their mode of formation is precisely the same. At this stage the body appears rather granular (Fig. 16), but when it has become compact in many cases it loses this appearance. In the hair cells the granular form is often retained, but is almost always lost in all other tissues. The constituent protein substance becomes homogeneous and vacuoles may appear in it (Fig. 23). In the inclusion bodies of the hairs vacuolation occurs in about one-half the cases, although the granular form may be retained, but in those of other tissues it occurs almost invariably. At no time could any indication of a limiting membrane be found.

If the host plant is kept under good growing conditions the process of formation of the rounded inclusion body occupies two or three days only. The condensation of the protein substance to give a more compact body and the process of vacuolation continues for some days after this. If, however, growing conditions are poor, as during the winter, the development of inclusions is retarded as is the development of macroscopic symptoms. For a long while large irregular masses of protein material move slowly about the cell. Usually they seem less granular than in the more rapidly growing plant, appearing to consist of aggregations of a more crystalline material. Ultimately these masses come together to give the typical inclusion body.

When the inclusion body is fully formed the activity of the cell contents is greatly diminished. Very little cytoplasm is visible; the peripheral lining layer is still present and streams feebly around the cell. Occasionally a few small protein particles are seen in it. Threads of plasm are thrown out across the vacuole, but only very rarely (Fig. 19). The inclusion body may move, but usually its motion is so slow as to be scarcely discernible. Often it settles down against the cell wall, frequently against the lower septum, and may then become somewhat flattened as the nucleus almost invariably does if in a similar position

(Figs. 18, 19, 32). A second inclusion may become evident in some of the hair cells (Fig. 20); this is a long colourless spike-like crystal (24). Its length is one-half to three-quarters that of the cell in which it lies apparently motionless. Occasionally two such crystals are present.

The cell contents now continue practically at rest for the space of several weeks, after which the large spherical body begins to crystallise out. Crystals are formed first on the surface, these move away from the body and gradually the whole mass breaks down. After a while the crystals, which give a strong protein reaction, are dispersed throughout the cell (Fig. 24). Then they gradually decrease in number until after about four or five months no sign is left of them or of any virus inclusion bodies. Presumably these crystals as well as the spike have been dissolved into the cell sap (Figs. 25, 33).

Size and distribution of the intracellular inclusions.

Significance has often been attached to the apparent association of nucleus and inclusion body, as they often lie in close proximity; it has been suggested that the latter might be a degeneration product of the nucleus. However, it has been shown that, owing to the confluence of several strands of cytoplasm in this area, particles tend to accumulate around the nucleus. Consequently the largest aggregations of protein material are often found here, the inclusion body being ultimately formed in close proximity with the cell nucleus. This is by no means a general rule. Any part of the cell may become the focus for the final agglomeration of all the protein particles, but it is usually the point of convergence of several strands of streaming cytoplasm. If the body be formed near the nucleus, it may move away later, or if it be formed independently of the nucleus it may join it later but possibly only for a while. Sometimes during its movement about the cell the body impinges on the nucleus causing a depression to appear in the surface of the latter; if it moves away again the nucleus resumes its former rounded contours. The nucleus itself appears to be totally unaffected by the virus disease.

Although both symptoms appear about the same time, the formation of intracellular inclusions bears no apparent relation to the development of chlorotic areas on the leaf. Bodies are formed in the green tissues equally abundantly as in the yellow tissues. Such bodies are contained by practically every hair of an infected leaf; possibly only the basal part of the leaf may show the mosaic, but the inclusions are equally prevalent in the hairs on the green area towards the tip. The inclusions are always larger than the cell nuclei, but their size varies and probably bears some

relation to the size of the containing cell and to the amount of cytoplasm present. The largest inclusions are found in the basal cells of the hairs where they often reach a diameter of 30μ. In the upper cells of the hair they are usually smaller (15–20μ), and they may be entirely absent from the distal cell. Although inclusion bodies occur with such frequency in the protective hairs they have never been observed in the glandular hairs. These each consist of a single stalk cell and a secretory head divided into quadrants. The latter are normally filled with protein material which might possibly obscure any virus inclusion.

In both the upper and the lower epidermis (Figs. 29, 30) inclusion bodies are formed in only a small percentage of the cells, not with nearly such frequency as they occur in the hairs. If one epidermal cell is found to be so affected, all the cells in its vicinity are found to contain such bodies. These patches of affected cells in no way correspond to either the green or the chlorotic areas; they occur indiscriminately in both. In the elongated epidermal cells covering a vein such bodies appear in a somewhat larger proportion of the cells; here again the affected cells are restricted to certain areas. The epidermal inclusion bodies are smaller than those of the hair cells, being only about 10μ in diameter.

In the palisade tissue inclusions are rather rare and when they occur are even smaller than in the epidermis, reaching only about 7μ in diameter. Here again affected cells are restricted to definite areas which, in the instances observed, were always below epidermal cells containing inclusion bodies. Similar bodies have been observed in the parenchymatous tissue below, but only very rarely indeed. So far no inclusions have been observed in vascular tissues.

The spherical inclusions and the spike-like crystals are by far most abundant in the tegumentary tissues, especially those near the vascular bundles.

Solanum lycopersicum.

The normal plant.

The leaf of *S. lycopersicum* is a more delicate structure than that of *S. nodiflorum* or *S. nigrum.* The lamina consists of a single layer of palisade tissue with two to three layers of spongy parenchyma below. The epidermis is not so thickly cuticularised as in *S. nodiflorum.* As in the latter the leaf bears innumerable hairs, but these are longer and finer. Their walls although thickened are not so much so as in the other species described, nor are they decorated with the minute papillae. Each hair is divided into four to six cells which taper very gradually to the apex.

Most of the hairs are of the protective type, the apical cells being conical in form. A few of them are glandular, a rounded secretory head replacing the conical cell of the more usual type of hair. The cells of the stalk are similar in all respects in both healthy and infected plants to those of the protective hair.

The individual hair cell is much more elongated than in the previously described forms (Fig. 34). The wall is lined with cytoplasm and many fine strands stream across the central vacuole. As in *S. nodiflorum* the contents of the cell are continually moving. Strands of cytoplasm disappear from view, others appear in new places; they anastomose and may fuse together completely or they may break away again. The nucleus is embedded in plasm which carries it around the cell. Sometimes it is suspended in the vacuole by fine strands, at others it rests against the cell wall or it may be rolled along the wall by the flowing of the plasm. The plasm contains mitochondria and minute globules of oil. Except in the very young leaves, there are no plastids present in the hair cells.

In many respects the histology and the cytology of *S. nodiflorum*, *S. nigrum* and *S. lycopersicum* are very similar. The chief disparity lies in the greater delicacy of structure throughout the latter.

The diseased plant.

Inclusions similar in form to those of *S. nodiflorum* occur in the tomato when it is attacked by aucuba mosaic. As in other hosts the first symptoms of the disease are not visible until several days have elapsed after infection (23). At about the time of appearance of macroscopic symptoms the cytoplasm becomes more conspicuous, its streaming is accelerated and innumerable minute particles appear in it; by successive fusions these increase in size (Fig. 35). They are carried about the cell by the plasm. By aggregation and fusion large protein masses are built up (Figs. 36, 37, 38). As they become larger, their speed of movement about the cell decreases. In the course of a few days, practically all the particles are contained within a single mass. This becomes more or less rounded, but its contours are often not so smoothly curved as in *S. nodiflorum*. Its substance condenses and the body gradually becomes more compact (Fig. 39). In the hair cells it usually retains a granular appearance, but in other tissues it may become more homogeneous. Vacuolation may occur as in *S. nodiflorum* and *S. nigrum*. Its chemical nature is similar to that of the bodies produced in these two species.

As in other hosts a spike-like crystal appears (Fig. 41). Very occasionally a third type of inclusion body is found which also occurs in

some of the other hosts examined, but only very rarely. It consists of large hexagonal crystalline plates or of irregular polygonal plates which appear to be built up from a number of hexagonal ones of varying sizes lying edge to edge (Fig. 40). In side view these are rectangular. On addition of weak acids they become striate. These inclusions appear to be identical with the striated bodies commonly associated with tobacco mosaic diseases (6). However, in this case they occur so abundantly in the diseased tissue as to be described as "characteristic products of the reaction of the cells to the presence of the mosaic virus"; whilst with aucuba mosaic if they are found at all it is only very occasionally and then only in isolated cells.

As in *S. nodiflorum* the inclusions persist for some weeks, the containing cell being practically at rest. Then the spherical inclusion body crystallises out (Fig. 41). After several months the inclusions begin to dissolve. Finally, all inclusions disappear and the cell is indistinguishable from one of an old but healthy plant.

Inclusions are formed almost as abundantly as in *S. nodiflorum* and are distributed very similarly, but usually they do not attain the same dimensions. Their diameter may reach about 25μ in the basal cells of the hairs, but usually they are considerably smaller. They are formed in most of the hair cells of an infected leaf irrespective of green and chlorotic areas. However, they are seldom found in the hairs on the stems and petioles. In the leaf epidermis and especially in the elongated cells covering the veins, patches of cells in both green and yellow parts may contain inclusion bodies as in *S. nodiflorum*. Again, bodies are found in the palisade tissue, but only very infrequently. When they do occur, several adjacent cells are always so affected. When, as occasionally happens, the leaves of the host assume the "fern-leaf" condition, intracellular inclusions are particularly well developed in the tegumentary tissues.

Nicotiana tabacum.

The normal plant.

The leaf of *N. tabacum* is more fleshy than those of the forms previously described. In transverse section it shows one or two layers of palisade tissue with about three layers of parenchymatous tissue below. It is enclosed by a thinly cuticularised epidermis, the surface of which is covered by hairs. Most of the hairs are glandular, each consisting of a stalk of about four elongated cells with a globular head made up of one to three cells which are filled with protein material and a bundle of crystals. The hairs may branch, each branch ending in such a globular

secretory head. A few are not of the glandular type and terminate in a conical cell. The walls of the hair are comparatively thin.

The cells of the stalk vary in size, the basal ones often being relatively large. They are elongated, tapering gradually to the globular head. The wall is lined with streaming plasm in which are embedded mitochondria, oil globules and frequently plastids. There appears to be more cytoplasm in these cells than in those of the previously described forms. The cell contents are in a continuous state of motion. Strands of cytoplasm are often thrown across the vacuole, but usually anastomoses are frequent and part of the cell may be occupied by a meshwork of streaming plasm. The nucleus is embedded in the cytoplasm and with the other inclusions it is carried about the cell (Fig. 42).

The diseased plant.

As in the other forms described, infection with aucuba mosaic produces on the leaves of tobacco a characteristic irregular mottling. There is, however, no apparent difference in the thickness and development of the leaves in the green and yellow areas. A sharp histological difference of this nature has been described as occurring in mosaic disease of tobacco (6, 10). No such arrested development has been observed in any of the hosts infected with aucuba mosaic and so far described (cf. *Hyoscyamus niger*). Intracellular inclusions are produced regularly in tobacco on infection with aucuba mosaic. They occur in many of the stalk cells of the hairs; in the secretory cells at the apex they have not been observed, but their presence might be obscured by the normal contents of these cells. They are found in localised areas of the epidermis scattered over the leaf irrespective of chlorotic and "healthy" tissues, and occasionally in the palisade tissue. Here again this disease shows differences from tobacco mosaic, for with the latter inclusions are stated usually to be limited to chlorotic areas where they are abundant in hairs, epidermis and palisade (6).

The mode of formation of these inclusions in all tissues is similar to that just described for the three *Solanum* species. At the end of the incubation period, the streaming of the cytoplasm quickens, minute particles appear in it and aggregate to form large masses (Figs. 43, 44, 45). Most usually the body rounds off and behaves as in *S. nodiflorum* (Figs. 45, 46), but fairly frequently it remains as a large irregular mass of granular protein material. This may become vacuolate. Later, a spike crystal is often formed (Fig. 46). After some weeks the body crystallises out (Fig. 47). It is not known whether these crystals ultimately dissolve

as, seven months after inoculation, they were still present in the cells. The chemical reactions of particles, inclusion body and the crystals to which it gives rise are similar to those already described for the inclusion bodies in other hosts infected with this disease. No other types of inclusion bodies were found in any of the tissues. No striated bodies were observed, but it cannot be said that these are never formed as they are seen only very rarely in any host infected with aucuba mosaic.

Hyoscyamus niger.

The normal plant.

In transverse section the healthy leaf shows a single layer of closely packed elongated palisade cells with innumerable chloroplasts. Below this are several layers of spongy tissue with well-marked intercellular spaces. The epidermis is only slightly cuticularised, the whole surface of the leaf lamina and petiole being covered with long hairs. The majority of these are glandular, the stalk consisting of from three to as many as eight or nine elongated cells and bearing a small globular secretory head of about three cells.

Each of the stalk cells is thin walled. The walls are lined with cytoplasm and the thin strands which flow across the vacuole often become enmeshed. Mitochondria and oil globules are carried about the cell by the flowing of the plasm, and plastids are embedded in the lining layer. The nucleus also is carried passively about the cell (Fig. 49).

The diseased plant.

Hyoscyamus is very badly affected by aucuba mosaic, seedlings often succumbing to the disease a few days after inoculation. The leaves show the characteristic mottling of aucuba mosaic, this symptom being unusually brilliant.

The cytological effects also are marked. At first the hair cells are affected in a similar way to those of the other hosts described. All movement within the cell is accelerated, then particles appear and circulate in the plasm (Fig. 50). They increase enormously in number and aggregate into large masses which lie apparently quiescent and usually against the cell wall. Occasionally these protein masses round off to give the typical form of inclusion body of aucuba mosaic in *S. nodiflorum* or *S. nigrum,* but usually there is no contraction of the mass and no rounding of its contours, the aggregations of particles being spread irregularly throughout much of the cell (Figs. 51, 52). They retain their granular form and may become vacuolate.

Often a spike crystal appears but no striated bodies. After a time the protein masses begin to crystallise out (Fig. 54). It is not known what would be the ultimate end of the protein masses and the crystals to which they give rise as the plants usually succumb completely to the disease. These inclusions are contained by all the hairs on the yellow areas, but seldom by those in the green tissues.

An attack of aucuba mosaic has a marked histological effect on the leaves of *H. niger* comparable to that of mosaic disease of tobacco. The chlorotic areas of the leaves are much thinner than are the healthy green areas of the plant. This is due to arrested development of the leaf tissues. The palisade cells are not elongated as in the normal leaf, and the intercellular spaces in the spongy tissue are poorly developed. The chloroplasts are destroyed or never develop. Irregular protein inclusions such as are formed in the hair cells are produced in practically every cell of the parenchymatous and epidermal tissues. No such inclusions are formed in the green areas of the leaf (Fig. 53).

Discussion.

The nature of the inclusion bodies.

Much has been written concerning the nature of intracellular inclusions produced as a result of infection with virus diseases of both plants and animals. Frequently a disease may produce several differing types of inclusion body. Some of these are similar to inclusions occurring in healthy plants and are obviously reaction products of the cell: the striate material accompanying tobacco mosaic and the spike crystal of aucuba mosaic of tomato are of this order. Possibly many of the bodies described as being associated with a particular virus disease are not produced as a direct result of infection; Klebahn (11, 12) described spirally twisted thread-like bodies, "scolecosomes," as occurring in the phloem cells of *Anemone nemorosa* infected with "Alloiophyllie," but later identical inclusions were found in healthy anemones.

However, it is to the rounded protoplasm-like inclusion that so much significance has been attached. Various theories have been advanced as to the origin and significance of this body. The views as to its nature have been summarised by Rivers (20) and Ludford (15) for the diseases attacking animals and by Henderson Smith (24) for those attacking plants. In both cases the theories fall into three main groups: it has been suggested that they are parasitic organisms causing the disease; that they are products of interaction of the host cell and the virus or the result

of cytoplasmic or nuclear reaction or degeneration; or that they consist of organisms combined with products of cellular reaction.

Protozoan organisms have often been described as occurring in association with virus diseases and have been presumed to be the causative agent. However, in a recent paper McLennan (17) describes a variety of *Leptomyxa reticulata* which is associated with a virus disease of hops. It occurs in some of the infected plants only and is regarded as a secondary invader; the resistance of the host having been lowered by the virus disease, the protomyxean form is able to enter the cells. Possibly other of the organisms described are such secondary invaders.

That the spherical bodies produced by aucuba mosaic are not organismal is shown by their mode of formation and their subsequent crystallisation and dissolution. It has been shown that they are formed entirely independently of the cell nucleus; their frequent association with it has already been explained. Indeed, with aucuba mosaic the nucleus of the host is entirely unaffected by the disease and in only one case in the plant kingdom, that of dahlia mosaic (7), has a definite invasion of the nucleus been shown. These bodies are apparently composed mainly of the products of cell reaction to the stimulus of the virus. Possibly they also contain the etiological agent of the disease, whatever its nature. The body is built up by the aggregation of particles of altered cytoplasm. Presumably the virus is dispersed throughout the cytoplasm, so that it is reasonable to suppose that it is contained within these particles.

However, the mode of formation of these bodies might account for much of the evidence which has led to the belief that they are living organisms. Many of the phenomena occurring in the living cell would be wrongly interpreted if observed only in fixed preparations. The fission figures that have been described and the presence of several bodies in one cell can easily be paralleled here (Figs. 21, 22, 38). After the rounding off there is no increase in size, rather there is a contraction, but in fixed preparations the variation in size from cell to cell might be regarded as evidence of growth of the body. Pseudopodia have been described also. The fusion of a larger and a small mass (Fig. 11) or an irregularly shaped body in process of formation (Fig. 15) or the breaking away of a portion of the body (Fig. 22) might easily be so interpreted in fixed preparations.

It seems probable that the intracellular inclusions of plants are of several different types as they are in animal diseases, and they may differ equally in their mode of origin. It is hoped that when more is

known of them, of the reaction of many hosts to one virus and a comparison is made of many viruses affecting the same host, that they will be of use diagnostically. At present but little systematic cytological work of this kind has been carried out with any one virus. Very many diseases have been studied cytologically, but only on isolated host plants. It is known that tobacco mosaic produces similar inclusions in *Nicotiana tabacum*, *Capsicum annuum*, *Lycopersicum esculentum*, *Physalis pubescens*, *P. franchetti*, *Petunia violacea*, *Hyoscyamus niger*, *Nicandra physoloides*, *Solanum laciniatum*, *S. miniatum* and *S. atropurpureum* but in *Nicotiana glutinosa* and *N. glauca* inclusions are not formed (8). Cellular inclusions have not so far been found in plants infected with cucumber mosaic. Aucuba mosaic of tomato in the hosts examined always tends to produce bodies of a certain type. These, however, may not always reach the same degree of development; in *S. nodiflorum* etc., they are usually rounded, whilst in *H. niger* they remain as irregular masses. A study is at present in progress of these same hosts infected with tobacco mosaic, but until the work is further advanced it is preferred to draw no parallels between the two diseases. The type of inclusion formed appears to be specific to the virus, the formation of the body being the response of the host cell to some peculiar characteristic of the particular virus. However, in certain cases, the host may modify slightly the form of the body. In view of Hoggan's results it seems improbable that cytological characteristics will always serve as a means to identification of a particular disease, but when one host is susceptible to several diseases they should serve as a means of differentiation.

Summary.

A description is given of the mode of formation of intracellular inclusions produced by aucuba mosaic of tomato in *Solanum nigrum*, *S. nodiflorum*, *S. lycopersicum*, *Nicotiana tabacum* and *Hyoscyamus niger*.

Soon after infection the rate of streaming of the cytoplasm is increased, then minute particles of protein appear in the cytoplasm which carries them passively about the cell. These particles aggregate and fuse to form large masses which are still carried passively but more slowly about the cell. These fuse until all the protein material is contained in one or occasionally more granular masses. In the three *Solanum* species examined this mass becomes rounded and it may lose its granular appearance and become vacuolated. In *N. tabacum* the body does not always round off and in *H. niger* it very seldom does so but remains as

an irregularly shaped granular mass which may, however, become vacuolate.

There is no evidence at any time of autonomous movement, the particles and the fully formed body being carried, as are the cell nucleus, mitochondria, etc., of the normal plant, in the cytoplasmic stream.

After the spherical body is formed a spike-like crystal appears in the cell.

The cell remains at rest for the space of several weeks. Often the rounded inclusion body and the nucleus are juxtaposed, but there is no special significance in this, it is merely the accidental result of the mode of formation of the body. Particles tend to accumulate where a number of strands of plasm meet; usually several strands converge on the nucleus.

Ultimately the body breaks down giving a number of protein crystals. After some months these dissolve. In *H. niger* the inclusion bodies are confined to the chlorotic areas where they are abundant in all tissues. In the other species studied they are distributed over green and yellow tissues. They are very abundant in the hairs, less so in the epidermis and very rare in the palisade and spongy tissues. In *H. niger* the development of the palisade tissue is arrested, in the other species the development is not so obviously affected although growth is retarded.

These inclusions appear not to be organismal in nature; they seem to be products of reaction of the host cell to the virus, but they may contain the etiological agent of the disease.

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PLATE XXV

Fig. 1 *a*.

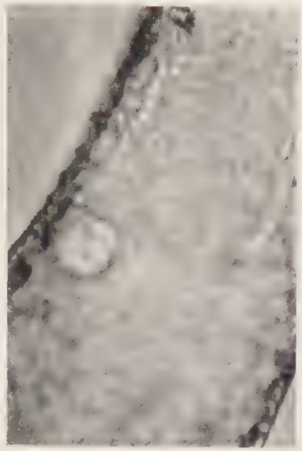


Fig. 1 *b*.

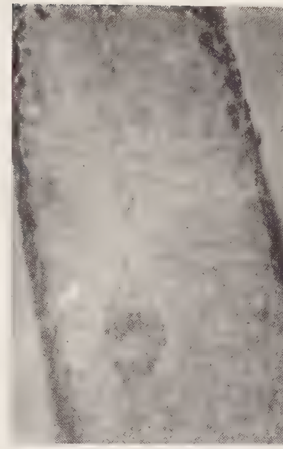


Fig. 2.

Fig. 3.

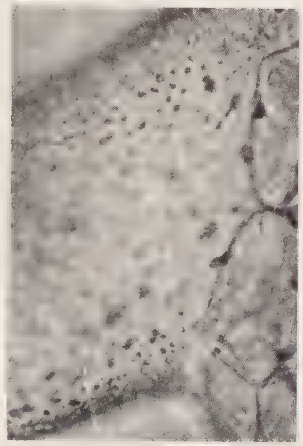


Fig. 4.

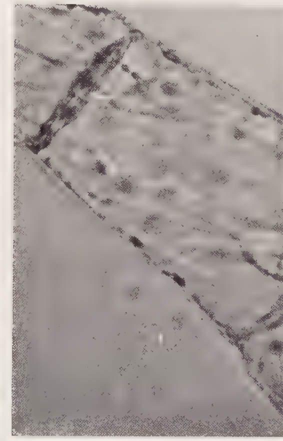


Fig. 5.

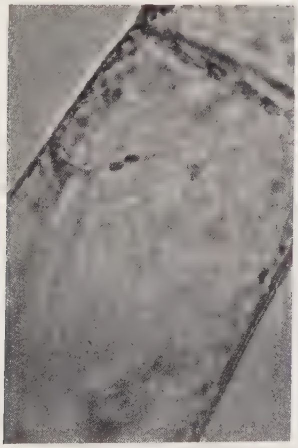


Fig. 6 *a*.

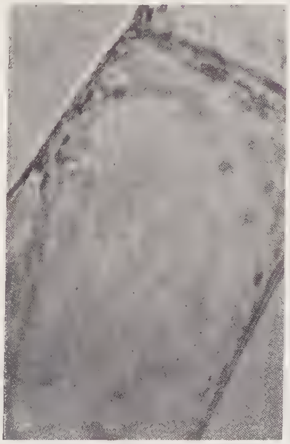


Fig. 6 *b*.

SHEFFIELD.—The Formation of Intracellular Inclusions in Solanaceous Hosts Infected with Aucuba Mosaic of Tomato (pp. 471–493).

Fig. 7 *a*.

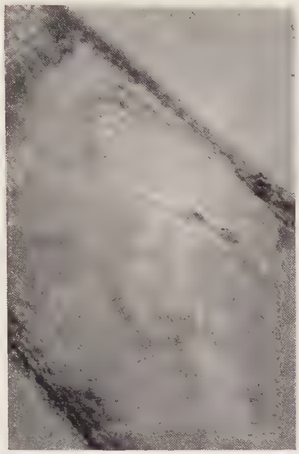


Fig. 7 *b*.

Fig. 7 *c*.

Fig. 7 *d*.

Fig. 7 *e*.

Fig. 8.

Fig. 9.

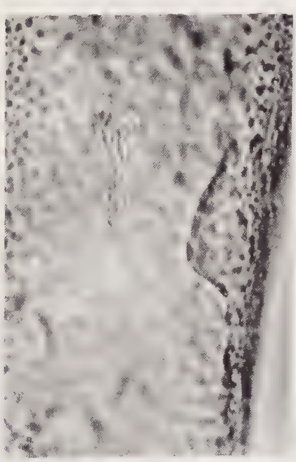


Fig. 10 *a*.

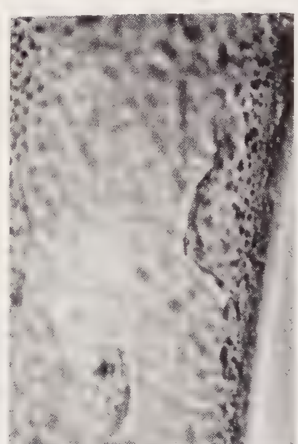


Fig. 10 *b*.

SHEFFIELD.—The Formation of Intracellular Inclusions in Solanaceous Hosts Infected with Aucuba Mosaic of Tomato (pp. 471–493).

EXPLANATION OF PLATES XXV—XXXIII

Figs. 1–20 are enlargements from single pictures of a cinematograph film demonstrating the effect of the disease on the cells of the hairs of *S. nodiflorum*. The film was taken using a Leitz No. 5 objective and a I ocular. The magnification on the negative was $\times 225$. The prints were enlarged three times, but in reproduction they have been reduced by $\frac{1}{3}$, giving a final magnification of $\times 450$.

All other figures were taken with a Leitz Micca camera. A Leitz 6L objective combined usually with a Leitz $\times 10$ periplanat eyepiece were employed giving a magnification on the negative of $\times 150$ at a tube length of 170 mm. The prints were enlarged $2\frac{1}{4}$ times and have been reproduced without further alteration. Magnification $\times 340$.

For Fig. 25 a Leitz periplanat ocular $\times 12$ was used—this gave a magnification of $\times 180$ on the negative. The print was enlarged $2\frac{1}{4}$ times increasing the magnification to $\times 400$.

All figures are taken from unstained living cells.

A mottled appearance across the cell in many of the earlier figures is due to the papillose thickening of the cell wall.

PLATE XXV.

Solanum nodiflorum.

Fig. 1 *a*. Part of a cell from the hair of a healthy plant. The nucleus is near the wall. Several fine strands of cytoplasm are flowing more or less parallel to the long axis.

Fig. 1 *b*. The same cell $2\frac{1}{2}$ minutes later. The nucleus has moved slightly out of focus. Most of the cytoplasm previously apparent has disappeared, and a very fine thread now runs obliquely across the vacuole.

Fig. 2. Part of hair cell of infected plant. Minute particles are visible in the cytoplasm, especially in that surrounding the nucleus.

Fig. 3. Larger particles are visible in the rather conspicuous cytoplasm. The nucleus is suspended in the vacuole by plasmic threads.

Fig. 4. Similar protein particles in the basal hair cell and in the epidermal cells.

Fig. 5. Numerous particles carried in the layer of plasm lining the wall and in strands crossing the vacuole.

Fig. 6 *a*. A similar stage to Figs. 3–5. Note two particles moving together along a strand of cytoplasm.

Fig. 6 *b*. 24 seconds later these two particles fuse.

PLATE XXVI.

S. nodiflorum, cont.

Fig. 7 *a–e*. Fusion of particles. Two particles are moving slowly along a thread of cytoplasm, a second pair approach rapidly behind them and all join together. 12 seconds elapse between *a* and *b*, 6 between *b* and *c*, 3 between *c* and *d*, and 3 between *d* and *e*.

Fig. 8. Numerous particles moving about the cell.

Fig. 9. Particles increase in size.

Fig. 10 *a* and *b*. A large plastic mass of protein material is drawn across the cell by a cytoplasmic strand, changing its shape as it travels. Other protein material is collected near the nucleus. 36 seconds elapse between *a* and *b*.

PLATE XXVII.

S. nodiflorum, cont.

Fig. 11. The nucleus is suspended in the vacuole by a number of strands of cytoplasm. Large aggregations of protein material are accumulating near it.

Fig. 12 *a*. As in Fig. 11, protein material is accumulating around the nucleus which is suspended in the vacuole. Large masses of this material are brought to the nucleus often only to be torn away again.

Fig. 12 *b*. However, 25 minutes later a considerable amount of fusion has taken place.

Fig. 13 *a*. The nucleus is flattened against the cell wall. Two masses of protein approach a third which is lying against the transverse septum.

Fig. 13 *b*. 2½ minutes later, these three masses have commenced to fuse.

Fig. 13 *c*. 2 minutes later, all the protein material has moved away from the transverse wall and is juxtaposed to the nucleus.

Fig. 13 *d*. After another 2 minutes, the fusion of the protein masses is apparently complete. (The nucleus has disappeared from view behind the developing inclusion body.)

Fig. 13 *e*. But 3 minutes later, the protein masses fall apart again.

PLATE XXVIII.

S. nodiflorum, cont.

Fig. 14 *a*. A mass of protein material is travelling along the cell wall towards two other masses which are slightly out of focus.

Fig. 14 *b*. 48 seconds later the first mass has changed its form in rolling along the wall and has passed slightly out of focus. The other two masses now begin to move towards it.

Fig. 14 *c*. 36 seconds later all three masses fuse just out of focus.

Fig. 15. The protein material is drawn into a single irregularly shaped mass.

Fig. 16. The mass becomes smoother in outline but retains its granular appearance.

Fig. 17. It becomes rounded and more homogeneous but vacuoles may appear in its substance. A few small particles still circulate in the cell.

Fig. 18. The completely developed inclusion body may lie apposed to the nucleus.

Fig. 19. The nucleus and a much vacuolated inclusion body are widely separated but each is resting against a cell wall. The cytoplasm still streams around the periphery of the cell and a single strand flows across the vacuole.

Fig. 20. A spike-like crystal appears in the cell.

PLATE XXIX.

S. nodiflorum, cont.

Fig. 21 *a–d*. Two spherical inclusion bodies have been formed in this cell. When apparently about to fuse they are drawn apart. Later they again approach each other.

Note also the movement of the developing body in the upper cell.

10 minutes elapse between *a* and *b*; 30 between *b* and *c* and 15 between *c* and *d*.

Fig. 22. Part of this inclusion body is about to break away.

Fig. 23. Vacuolate inclusion bodies.

Fig. 24. After some weeks the bodies break down into protein crystals.

Fig. 25. Six months after inoculation all inclusion bodies have disappeared from the cells.

PLATE XXX.

Solanum nigrum.

Fig. 26. The basal cell of the hair of a normal plant. The nucleus, containing nucleoli, is suspended in the vacuole by numerous cytoplasmic strands which radiate to the cell wall. Plastids are embedded in the parietal plasm.

Fig. 27. An early stage in the development of inclusion bodies in an infected plant. Innumerable small particles of protein material have been deposited on the surface of the nucleus.

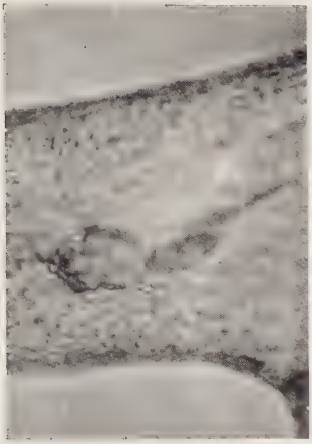


Fig. 11.

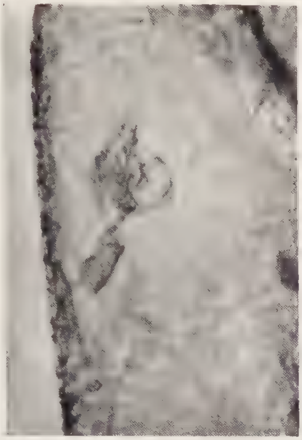


Fig. 12 *a*.

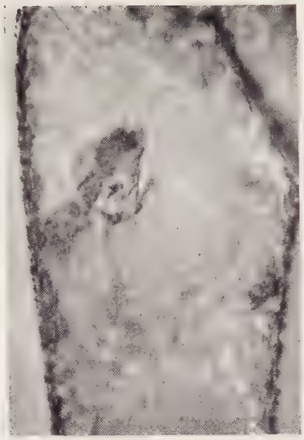


Fig. 12 *b*.

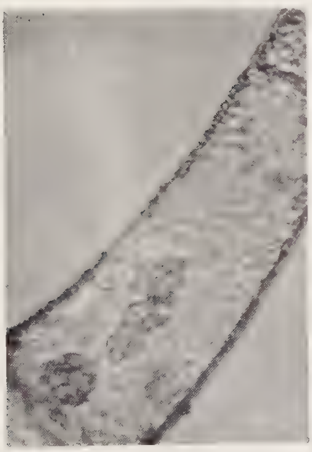


Fig. 13 *a*.

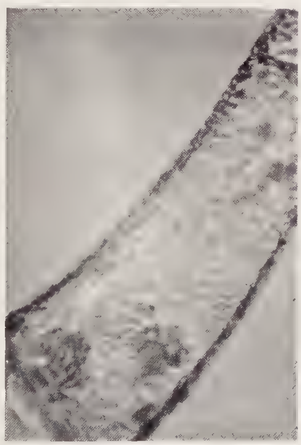


Fig. 13 *b*.

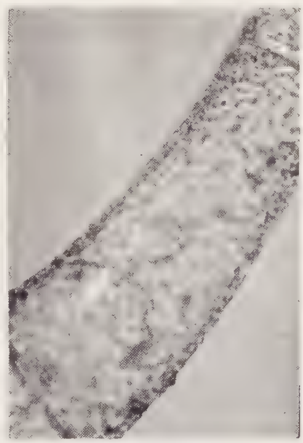


Fig. 13 *c*.

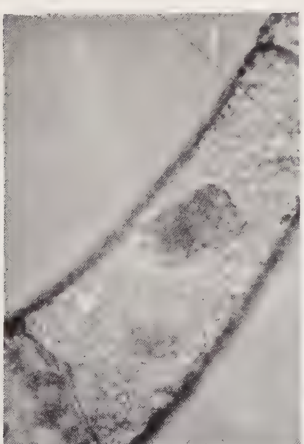


Fig. 13 *d*.

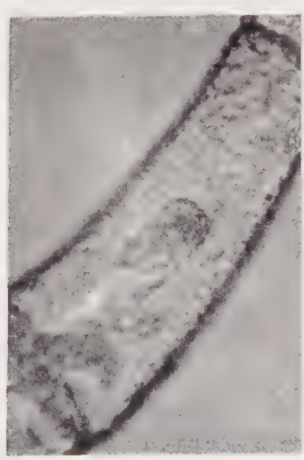


Fig. 13 *e*.

SHEFFIELD.—The Formation of Intracellular Inclusions in Solanaceous Hosts Infected with Aucuba Mosaic of Tomato (pp. 471–493).

Fig. 14 *a*.

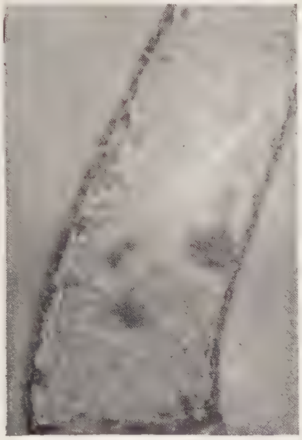


Fig. 14 *b*.

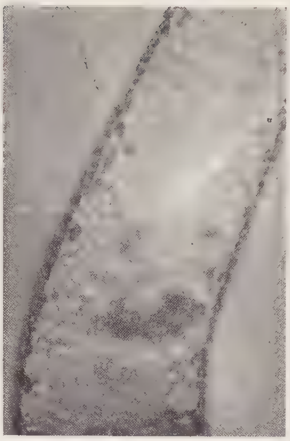


Fig. 14 *c*.

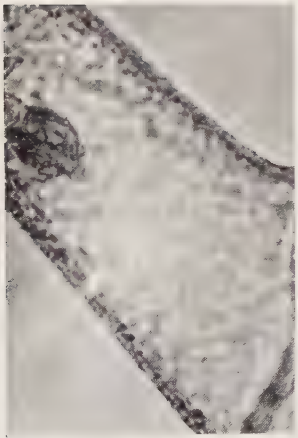


Fig. 15.

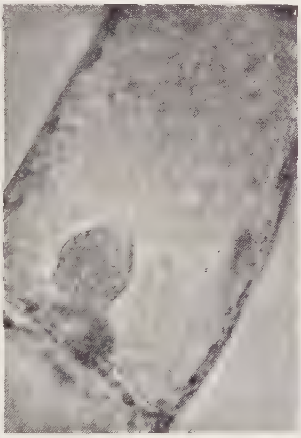


Fig. 16.

Fig. 17.

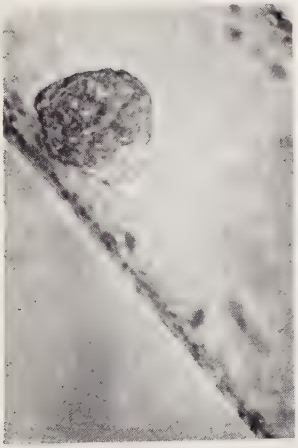


Fig. 18.

Fig. 19.

Fig. 20.

SHEFFIELD.—The Formation of Intracellular Inclusions in Solanaceous Hosts Infected with Aucuba Mosaic of Tomato (pp. 471–493).

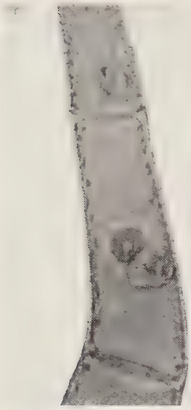


Fig. 21 *a*.


Fig. 21 *b*.


Fig. 21 *c*.


Fig. 21 *d*.

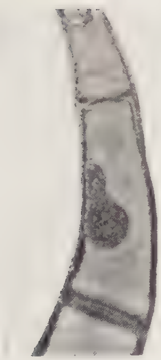


Fig. 22.

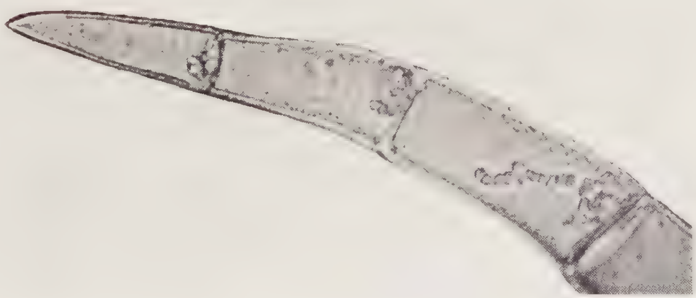


Fig. 24.


Fig. 23.

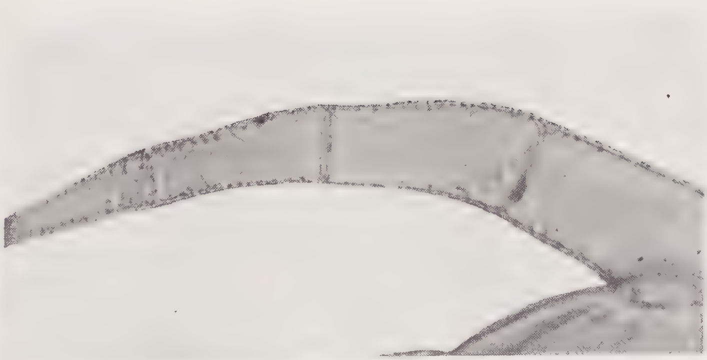
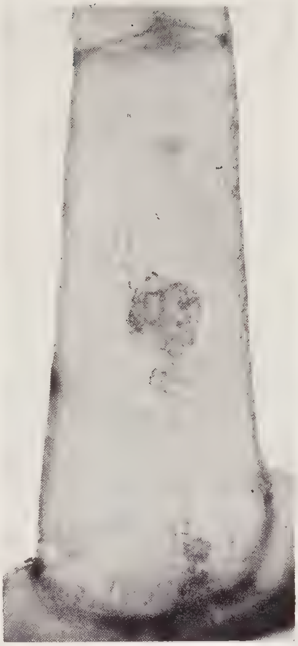


Fig. 25.

SHEFFIELD.—The Formation of Intracellular Inclusions in Solanaceous Hosts Infected with Aucuba Mosaic of Tomato (pp. 471–493).

Fig. 26.

Fig. 27.



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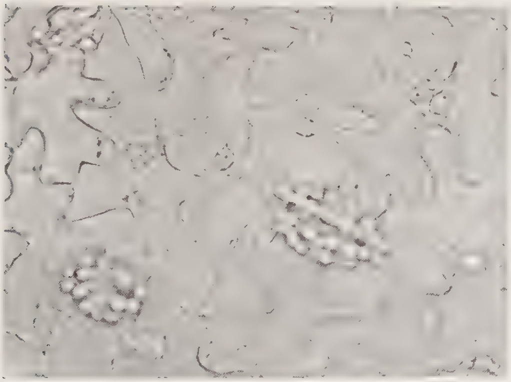

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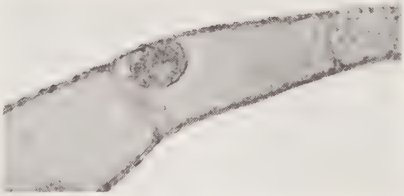

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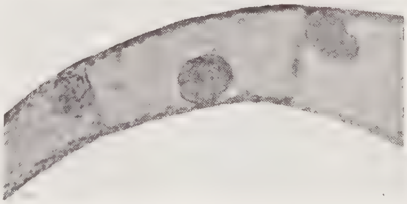

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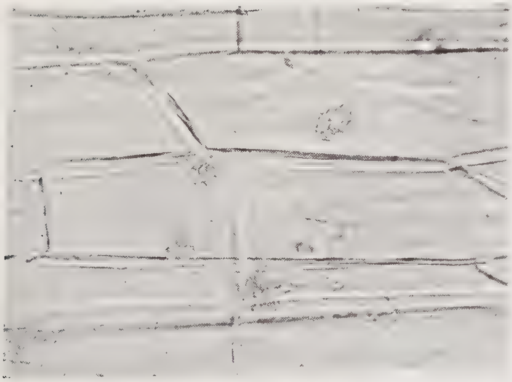

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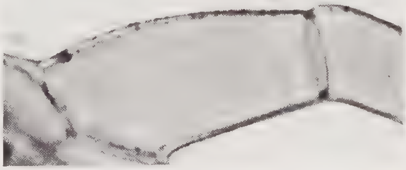


Fig. 33.

SHEFFIELD.—THE FORMATION OF INTRACELLULAR INCLUSIONS IN SOLANACEOUS HOSTS INFECTED WITH AUCUBA MOSAIC OF TOMATO (pp. 471–493).

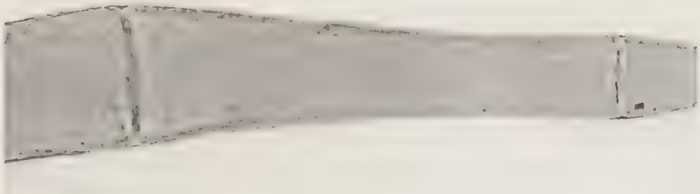
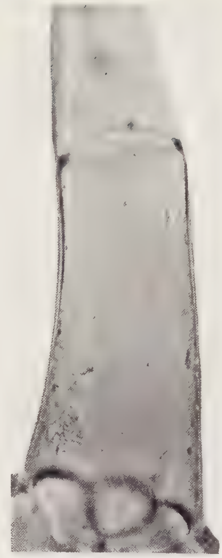


Fig. 34.



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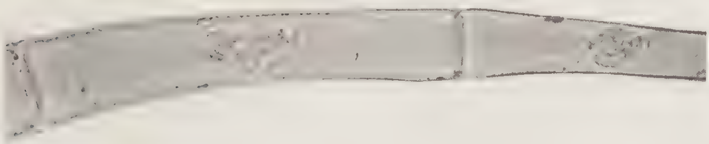

Fig. 36.

Fig. 37.

Fig. 40.

Fig. 38.

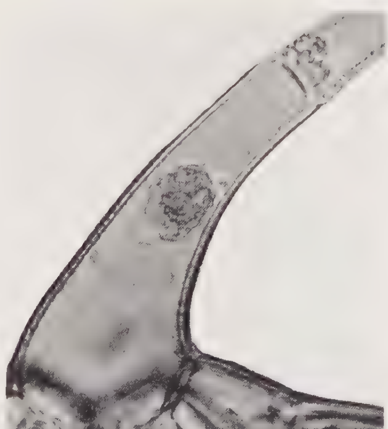

Fig. 39.

Fig. 41.

SHEFFIELD.—The Formation of Intracellular Inclusions in Solanaceous Hosts Infected with Aucuba Mosaic of Tomato (pp. 471–493).

Fig. 42.

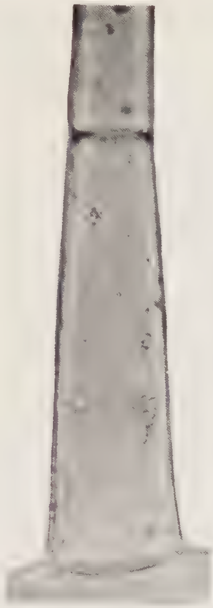

Fig. 43.

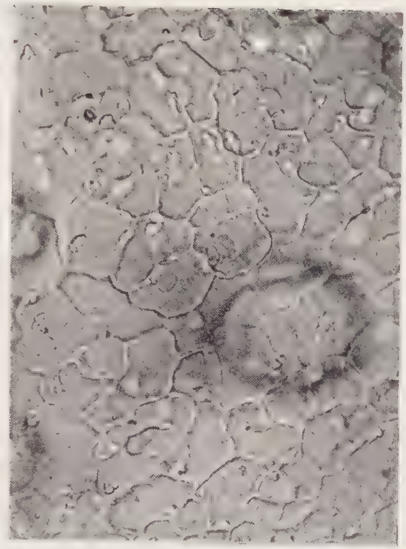

Fig. 48.

Fig. 44.

Fig. 45.

Fig. 46.

Fig. 47.

SHEFFIELD.—THE FORMATION OF INTRACELLULAR INCLUSIONS IN SOLANACEOUS HOSTS INFECTED WITH AUCUBA MOSAIC OF TOMATO (pp. 471–493).

Fig. 49.

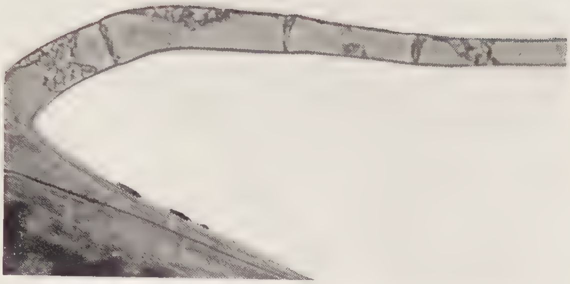

Fig. 51.

Fig. 50.

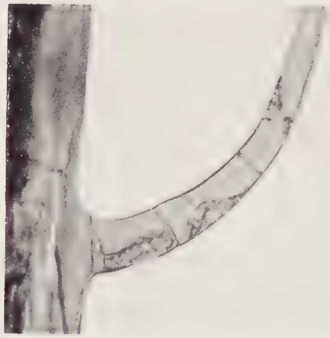

Fig. 52.

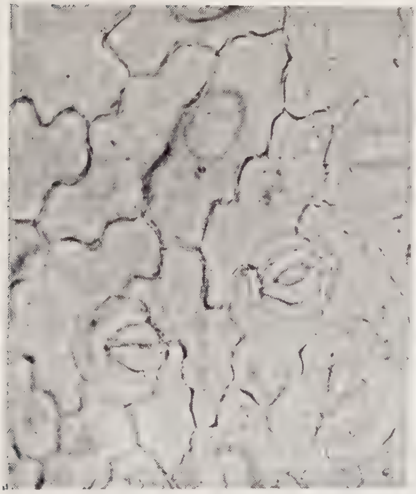

Fig. 53.

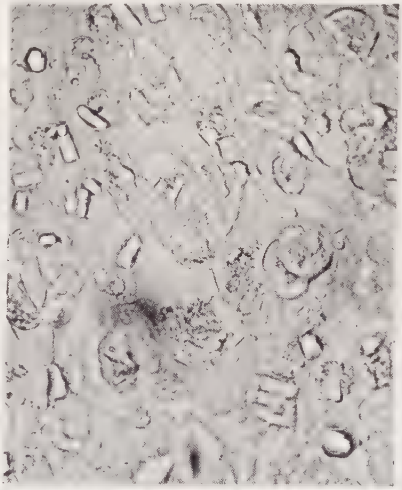

Fig. 54.

SHEFFIELD.—The Formation of Intracellular Inclusions in Solanaceous Hosts Infected with Aucuba Mosaic of Tomato (pp. 471–493).

Fig. 28. Large aggregations of protein material circulate in the cell.

Fig. 29. Developing inclusion bodies in the epidermal cells.

Fig. 30. Inclusion bodies developing in the epidermis from above a vascular bundle.

Fig. 31. A developing inclusion body is closely apposed to the nucleus.

Fig. 32. Fully formed inclusion bodies.

Fig. 33. After several months, the inclusion bodies have disappeared from the cells.

PLATE XXXI.

Solanum lycopersicum.

Fig. 34. A cell from the hair of a healthy plant. The nucleus is suspended in the vacuole by fine strands of cytoplasm.

Fig. 35. Soon after infection small protein particles circulate in the cytoplasmic strands. They have already formed one aggregation which is approaching the nucleus.

Fig. 36. Several aggregations circulate in the cell and fuse.

Fig. 37. Protein masses moving about the cell.

Fig. 38. The particles have all been drawn into two masses one of which rests against the nucleus.

Fig. 39. A fully formed inclusion body lying against the nucleus.

Fig. 40. Striate material is occasionally formed.

Fig. 41. The spherical body has crystallised but the spike crystal is still present.

PLATE XXXII.

Nicotiana tabacum.

Fig. 42. Part of a hair cell of a normal plant. The nucleus lies against the thin cell wall. Numerous plasmic threads cross the vacuole. Plastids are embedded in the parietal plasm.

Fig. 43. Soon after infection small aggregations of protein particles move about the cell. A number of these are collected near the nucleus.

Fig. 44. Larger protein masses move about the cell. The majority are collecting together near the nucleus.

Fig. 45. In the upper cell most of the protein material is now contained in one large irregular mass. A fully formed body lies apposed to nucleus and wall in the lower cell.

Fig. 46. The cell contains a granular, vacuolate inclusion body, a spike like crystal and the nucleus.

Fig. 47. A small glandular hair. Inclusion bodies which were formed in the stalk cells, have now crystallised.

Fig. 48. The bodies formed in the epidermis have crystallised.

PLATE XXXIII.

Hyoscyamus niger.

Fig. 49. Cells from the apex of a glandular hair of a normal plant. The stalk cells each contain streaming cytoplasm, a nucleus and plastids.

Fig. 50. Soon after infection. A few minute particles have appeared in the cytoplasm.

Figs. 51 and 52. Large aggregations of protein material lie against the cell walls.

Fig. 53. The epidermis from the green area of the leaf is exactly like that of a normal leaf. The cells contain cytoplasm and nucleus but no inclusion bodies.

Fig. 54. In the chlorotic areas of the leaf, the epidermal cells are smaller. They contain large masses of protein material which crystallise out.

(*Received March 10th*, 1931.)

SOME EXPERIMENTS ON INOCULATING METHODS WITH PLANT VIRUSES, AND ON LOCAL LESIONS

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(With Plates XXXIV–XXXVI.)

The work of Holmes (1929) has shown that in the case of tobacco mosaic a light rubbing of the virus over the leaf is a much more effective method of inoculation than scratching with a needle. He was able to demonstrate this by working with plants (*Nicotiana rustica*, *N. glutinosa*, etc.) on the leaves of which necrotic local lesions were formed at the points of entry of the virus, the numbers of these lesions affording a visual demonstration of the degree of effectiveness[1] of the various methods of inoculation. He also pointed out that these local lesions constituted a symptom of primary infection of a very definite character, which was entirely or almost obscured by the needle-scratching method of inoculation.

It is obvious that it is of considerable importance for future work on plant virus diseases to know how generally applicable these results of Holmes may be. Is the light rubbing method of inoculation, in which no visible wound is made on the inoculated leaf, the best for other virus diseases than tobacco mosaic, and does it apply for plants with smooth leaves as well as hairy? Also are local lesions formed at the points of entry in the case of other virus diseases, and if so are these local lesions of characteristic form, and are they likely to be of aid in virus differentiation, or particularly in developing quantitative methods such as Holmes is developing for tobacco mosaic?

A critical review of the literature affords indications that Holmes's methods might very well be applicable to many virus diseases. Even with viruses known to be highly infectious, by far the largest number of experiments recorded in the literature have not given 100 per cent.

[1] It might be claimed, of course, that if a needle scratch successfully introduces virus in one single place it is just as "effective" in the end as a method which introduces virus in 100 places. Holmes's point, however, was that the needle scratch is not the *type* of wound which is favourable for the entry of the virus.

infection. An infection of 60 to 80 per cent. has usually been regarded as extremely satisfactory, and little attention has been paid to the cause of failure in the cases where the disease was not contracted.

The indications are still more suggestive when one comes to review the experimental work on the less infectious virus diseases. In many of these cases it has been found that the rubbing of diseased material over the surfaces of the healthy leaves to be inoculated is very decidedly superior to pricking or scratching with a needle, or to injection of viruses by capillary tubes or hypodermic syringes.

Thus Reddick and Stewart (1919), working with bean mosaic, found that it was practically impossible to get infection by needle pricking or hypodermic syringe, but they did get a high percentage of infection by rubbing the under sides of the young leaves with diseased leaves rolled together and crushed. Fajardo (1930) also used a modification of the rubbing method with success.

Schultz and Folsom (1923), in their work with potato virus diseases, speak of the needle-prick method "which the writers long ago discarded as useless." They also found that the most satisfactory method of transmission was the rubbing of a considerable area of leaf surface with the virus-bearing material, though they usually did it so severely that the leaves inoculated were mutilated in the process.

Gardner and Kendrick (1921), working with turnip mosaic, and also with soy bean mosaic, got better results and a shorter incubation period when leaves were rubbed with crushed diseased material than when the virus was pricked and scratched into them. Other examples could also be cited where the rubbing method was claimed to give the best results, although in most cases the rubbing was apparently done hard enough to wound the inoculated leaves considerably.

There have been a few cases where workers have claimed to get the best results without visible wounding. Clinton (1915) considered the touch method the best for transmitting tobacco mosaic, and Fromme, Wingard and Priode (1927) found that transmission of ring spot of tobacco was accomplished most readily when the leaves of trial plants were merely swabbed with the inoculum.

On the other hand, in at least one instance (Brandes (1920) working with sugar-cane mosaic) transmission by rubbing has not succeeded, whereas hypodermic needle injection into the growing point has succeeded. Kunkel (1924), however, later obtained transmission of sugar-cane mosaic by the rubbing of leaves with diseased material. Again, the only successful mechanical inoculations with curly top of sugar beet

which have so far been reported have also been needle inoculations into the crowns of young beets (Severin, 1924).

Not only from these, but from many other published papers, the conclusion can be drawn that the most general methods for mechanical inoculation of plant virus diseases in use at the present time are the needle-scratch and needle-prick methods for the very infectious viruses[1]; "leaf mutilation," which is a severe form of rubbing, for less infectious viruses; and in some cases hypodermic needle injection for the most refractory cases. It is evident that there is very little of a scientific basis underlying these methods. The prevailing idea seems to be, at least to some extent, that the more a plant is wounded in presence of the virus the more chance for infection there will be, and inoculating methods are often correspondingly severe.

When working with the spotted wilt disease of tomatoes, which is normally transmitted in South Australia by a species of thrips, J. G. Bald and the writer, in some work as yet unpublished, found that although the disease was very difficult to transmit mechanically by the usual methods, it could readily be transmitted by the use of Holmes's rubbing method, in which no visible wound was made. This aroused interest in the general question of inoculating methods, so that when the occasion presented itself of working for a few months at Wisconsin University, the present writer took the opportunity to test some of Prof. James Johnson's viruses in this respect also, and the interest and co-operation of Prof. Johnson in the work are gratefully acknowledged herewith. Not only was the rubbing method of inoculation tested against needle scratching, but a watch was kept for local lesions of the viruses on various host plants, in case these might become of value in future quantitative work. The time available was comparatively short and all aspects of the work could not be followed to a conclusion, but enough has been done to verify Holmes's main contentions in the case of Johnson's viruses, and to indicate the desirability of other workers carrying on further tests on inoculating methods, both with these and still other virus diseases.

[1] Lately the rubbing method of inoculation, with or without wounding of the epidermis, has been adopted by several workers (Valleau and Johnson, 1930; Bewley, 1930).

COMPARISON OF INOCULATING METHODS WITH SIX OF JOHNSON'S VIRUSES.

The six viruses which were available were those of:

Cucumber mosaic (Cucumber virus 1).

Spot necrosis of tobacco (considered by Johnson (1929) to be potato rugose mosaic).

Ring spot of tobacco (ring spot of Fromme, Wingard and Priode).

Petunia mosaic (Petunia virus 1).

Yellow tobacco mosaic (Tobacco virus 6).

Ordinary tobacco mosaic (Tobacco virus 1).

Since these are all rather infectious viruses it was decided that the best comparison of methods of inoculation could probably be made with diluted samples. Johnson and his co-workers, of course, obtain 100 per cent. infection regularly with these viruses undiluted, and often when diluted 1 : 1000 or more, using the needle method of inoculation. It was only by using considerably higher dilutions, therefore, that the relative efficacy of the two methods of inoculation *per se* could be compared.

Table I.

Showing the infections resulting from inoculating tobacco seedlings with diluted viruses by the needle-scratch and by the rubbing methods.

Virus used	Dilution	No. of plants inoculated	No. of plants infected by Needle scratches	Rubbing method
Cucumber mosaic	1 : 1000	10	2	10
,, ,,	1 : 10,000	10	0	6
Spot necrosis	1 : 1000	10	10*	10*
,, ,,	1 : 10,000	10	6*	10*
Tobacco ring spot	1 : 100	10	9	10
,, ,,	1 : 1000	10	0	10
,, ,,	1 : 10,000	10	0	6
Petunia mosaic	1 : 1000	10	1	9
,, ,,	1 : 10,000	10	1	4
Yellow tobacco mosaic	1 : 10,000	10	10	10
,, ,, ,,	1 : 100,000	10	4	10
,, ,, ,,	1 : 1,000,000	10	0	9
Ordinary tobacco mosaic	1 : 10,000	10	10	10
,, ,, ,,	1 : 100,000	10	6	10
,, ,, ,,	1 : 1,000,000	10	0	8

* These figures include plants which developed only the "mottle" form of infection.

Table I gives the results of a comparison of the standard method of needle scratching and pricking with the rubbing method of inoculation for diluted samples of the above six viruses. The needle-scratch method

consisted of making ten scratches and ten pricks (a number directly into the midrib) on each of two leaves of young tobacco seedlings, with a needle round whose point was wrapped a little absorbent cotton to hold more virus. The rubbing method consisted of rubbing two leaves of the seedlings gently but firmly over their whole surface with a glass spatula (described below) dipped in virus, no visible wound being made on the leaves.

It is immediately evident from Table I that the rubbing method of inoculation gave more consistently successful infection in every case. Moreover it appears to be so much superior to needle scratching as a method of inoculation at high dilutions that it appreciably extends the limits of what are usually considered the maximum dilutions possible. The highest dilution at which consistent infection can still be obtained will depend very largely on the area of leaf surface wiped with the virus. Thus when larger plants than the seedlings used in Table I were inoculated with tobacco mosaic virus diluted 1 : 1,000,000, 100 per cent. infection was secured in each of three similar experiments. This naturally raises the question as to proper standardisation of inoculation technique in order to make dilution tests comparable.

Table II.

Showing the average number of local lesions per leaf resulting when tobacco plants were inoculated by the needle-scratch method and by three different degrees of hardness in the rubbing method of three comparable leaves per plant.

Exp.	Virus used		10 needle scratches	Light rubbing	Medium rubbing	Hard rubbing
1	Yellow tobacco mosaic	1 : 10,000	2	5	55	40
2	,, ,, ,,	,,	2	8	22	66
3	,, ,, ,,	,,	4	48	262	189
4	,, ,, ,,	,,	6	76	283	168
5	Spot necrosis	1 : 1000	0	31	138	39

Another way of comparing the two methods of inoculation is by counting the local lesions (see next section) which develop on the inoculated leaves. The numbers of lesions formed in any experiment will depend, among other things, on the concentration of the virus used, and on the size of the leaves inoculated. In each experiment plants as uniform as possible were used, and the virus was diluted 1 : 1000 or 1 : 10,000 to have the lesions separated sufficiently for reasonably accurate counting. Three arbitrary degrees of hardness of rubbing were tested, in none of which were any visible wounds produced on the leaves. Comparisons can only be made, of course, between the four methods of

inoculation done in a single experiment on uniform plants and with the same virus, and not between separate experiments. The results of several experiments are shown in Table II, from which it is evident that a single medium-firm rubbing of the virus over the leaves to be inoculated gave the most satisfactory results in the case of the two viruses tested. All the rubbing methods, it is to be noted, produced many more successful points of entry for the virus than did the needle-scratch method.

A number of comparisons between needle scratches and rubbing were also made on different halves of the same leaf. It was noticeable how often the successful infection points along the needle scratches were situated at the very end of the scratches where the pressure would have been lightest (Plate XXXIV, fig. 2). The reason for the superiority of the rubbing method over the needle-scratch method for inoculating with dilute viruses is quite evident from this figure.

Methods. In the experiment in which different degrees of hardness of rubbing were being compared, the inoculations were done by rubbing the diluted virus over the leaves with a piece of muslin wrapped round the forefinger. There is a delicacy in the forefinger which cannot quite be attained by the use of any artificial instrument. When many viruses are being worked with, however, and especially very infectious viruses such as the tobacco mosaics, it is undesirable to get virus on the fingers, since continual disinfection of the hands is tedious and difficult to perform.

For general inoculation work flat glass spatulas were made, and were found to be very satisfactory. After pressing out the expanded portion to a width of about 1 cm. and at an angle of about 135° to the handle, the underside is ground flat on a carborundum wheel or on carborundum paper. This gives a very good rubbing surface, which in comparative experiments yielded results as good, or even slightly superior, to those produced by the muslin wiping method. The leaf which is being rubbed with the spatula dipped in virus is supported by the left hand, the fingers of which are protected from contamination by a small square of clean paraffin paper held under the leaf (Plate XXXIV, fig. 1). The glass spatulas are sterilised after use, along with the mortars and pestles, in the steam steriliser.

A very quick and satisfactory way of making ordinary inoculations is to wipe the leaves with the pestle which has just ground up the inoculum in the mortar, the leaves again being supported on a square of paraffin paper.

All these methods, in which the leaves inoculated were merely rubbed with the virus without producing any visible wound, gave a higher

percentage of successful infection, and the disease had, on the average, a shorter incubation period, than when the needle-scratch method was used.

The local lesions.

One of the great advantages of the rubbing method—an advantage which Holmes has already pointed out—is that it enables the symptoms (when such are formed) of the "primary phase" of infection to be observed. These "local lesions[1]," as Holmes has called them, are often very characteristic, and are of considerable additional value in the total clinical or symptom picture of the disease. It is often quite impossible to distinguish them when the needle-scratching method of inoculation is employed.

They have, moreover, the additional advantage of being visible in many cases several days earlier than systemic symptoms, and when a large amount of work is being done with one virus it is usually found from experience that they are quite as reliable an evidence of infection as the systemic symptoms which develop later, and in some experiments may enable the time of keeping the plants to be reduced appreciably. Thus the local lesions of yellow tobacco mosaic and spot necrosis on tobacco may be visible in from two to three days under certain conditions.

Constancy of form of local lesions for a particular virus. The results of numerous experiments indicate that on a given host plant under given environmental conditions the primary lesions (in cases where such are formed) are definite and constant in character for a particular virus. The lesions formed by spot necrosis on *N. rustica* were found to be of the same concentric circle type, whether the source of inoculum was from infected tobacco, *N. rustica* or *N. acuminata*. Similarly the lesions on tobacco were found to be the same, whether the source of inoculum came from tobacco, or from either of the other two species of *Nicotiana*. The same was found to apply in the case of the faint yellow-blotch local lesions of yellow tobacco mosaic on tobacco, which were found to be constant in character, whether the inoculum was derived from tobacco, from *N. acuminata*, *N. rustica*, or other host plants of this virus.

When two viruses were inoculated one after another on the same

[1] The term "primary lesions" is perhaps rather more suggestive of the nature and position of these symptoms, developing as they do in localised spots at the points of infection on the inoculated leaves, but since Holmes mainly used the term "local lesions" both terms are employed in this paper.

leaf, or when a mixture of two viruses was inoculated on to a leaf, the primary lesions characteristic of each virus were both produced—provided the viruses were used in sufficient dilution to allow independent development at the successful points of entry for some time before coalescence due to proximity took place. Thus, when spot necrosis was inoculated on to the leaves of tobacco or *N. rustica*, and yellow tobacco mosaic was inoculated immediately following (the former virus being used in a dilution of 1 : 1000, and the latter in a dilution of 1 : 10,000), after some three to four days the circle primary lesions characteristic of the former virus, and the faint yellow-blotch primary lesions characteristic of the latter, both developed on the inoculated leaves. After a day or two, as virus "colonies" close to one another enlarged and met, necrotic spots were formed, and occasional wholly necrotic spots in the early stages were interpreted as cases where the infection points of the two viruses lay so close together that they intermingled almost at once.

Similarly if a leaf is inoculated with both tobacco mosaic and yellow tobacco mosaic, diluted 1 : 10,000, and three days afterwards the leaf is removed and treated by the iodine method (*v. infra*), the local lesions of the two viruses are clearly visible intermingled, those of the yellow tobacco mosaic having inhibited starch formation much more than those of the ordinary tobacco mosaic.

Effect of temperature on the primary symptoms. Temperature has a very marked effect on the development of the primary lesions, as is well shown in Plate XXXVI.

Ordinary tobacco mosaic, which forms small 1–2 mm. necrotic spots on *N. glutinosa* leaves at temperatures near 21° C., forms much larger and more quickly spreading necrotic spots at 28° C., while at 35° C. there is no necrosis at all, the inoculated leaves showing merely faint yellow-blotch primary lesions (Plate XXXVI, fig. 1). (It is an interesting fact that at the two lower temperatures, where necrotic local lesions are formed, systemic invasion of the plant does not occur, whereas at the higher temperature where no necrosis is produced on the inoculated leaves systemic infection of the plant rapidly follows.)

In the case of spot necrosis on tobacco, or on *N. rustica* and *N. acuminata* as illustrated in Plate XXXVI, figs. 2 and 3, the primary lesions are small necrotic spots at 21° C., concentric, less severely necrotic circles at 28° C., and nothing at all at 35° C. There is reason to believe that 35° C. is above the upper temperature limit for the development of spot necrosis, whereas ordinary tobacco mosaic and yellow tobacco mosaic still develop at this temperature.

Other factors influencing primary lesions. The species of host plant on which the virus is inoculated, of course, determines the form of the primary lesions to a considerable extent. Yellow tobacco mosaic forms faint yellow-blotch local lesions on tobacco and some other plants, but necrotic-spot local lesions on *N. glutinosa*, *N. repanda*, etc. There is, however, a general tendency for the local lesions of a particular virus to be of a particular type. Thus the local lesions of yellow tobacco mosaic appear to be of the yellow-blotch type whenever the host is not so hypersensitive that necrosis sets in, and the local lesions of spot necrosis tend to be of the concentric circle type on all hosts except a few which are again so sensitive that necrosis results.

Cucumber mosaic, on the other hand, has given no visible primary lesions on *N. tabacum*, *N. rustica*, *N. glutinosa*, and several other plants tested, although faint but distinct yellow-blotch local lesions, gradually becoming necrotic, developed on *N. sanderae*. Quite definite and distinct primary lesions developed on the leaves of sugar beet, however.

It remains to be seen whether such other factors as light, age, or condition of the plant inoculated, etc., also have an influence on the development of the primary lesions.

The number of lesions formed per leaf when a virus is rubbed uniformly over the surface varies considerably with the age of the leaf. When yellow tobacco mosaic diluted 1 : 10,000 was wiped over three leaves of medium-sized tobacco plants it was always found that the older of the inoculated leaves developed many more local lesions than did the younger. This is brought out by the figures in Table III.

Table III.

Showing the number of local lesions produced when three leaves[1] *of tobacco plants were wiped with yellow tobacco mosaic virus diluted* 1 : 10,000.

	No. of local lesions on		
Plant no.	Youngest leaf	Middle leaf	Oldest leaf
1	185	240	360
2	100	248	502
3	156	171	239
4	82	147	276
5 (10 needle scratches)	2	4	7
6 (10 needle scratches)	4	5	8

This is just the opposite to what Holmes found for the necrotic local lesions of tobacco mosaic on *N. glutinosa*. He says "the young leaves

[1] All three leaves in each case were of approximately the same size, being about four inches long; if anything the younger leaves were slightly larger than the older.

tend to produce more local lesions when inoculated with the same source of virus as neighbouring older leaves."

Commenting briefly on this work with local lesions, then, it is evident that when a virus is sufficiently dilute to produce well-separated points of infection, the multiplication of the virus which takes place in the tissues at these points may lead to the production of definite local symptoms, characteristic for the particular virus used. These symptoms may vary to some extent with the plant inoculated, and with temperature and other environmental conditions, but are constant under constant conditions. When a virus does not produce visible symptoms at the site of inoculation on one species of plant, it may do so upon another. Since the production of these local lesions offers hope of developing improved quantitative methods for virus work it would seem fully worth while to determine, when possible, the most suitable host plant and the most suitable environmental conditions for production of definite, easily visible, local lesions for any particular virus.

SPREAD OF THE VIRUS IN NON-NECROTIC LOCAL LESIONS.

It was found during the course of the above experiments that yellow tobacco mosaic was particularly well suited for demonstrating the infection centres obtained by different methods of inoculation, and also for following the spread of the virus to some extent, in spite of the fact that it causes no visible necrosis on tobacco.

The points of entry of the virus on the inoculated leaves become visible to the naked eye two or three days after inoculation (at a temperature of 28° C.) as rather faint, yellowish, indefinite spots in the normal green of the leaf (Plate XXXIV, fig. 3). For convenience, as well as for consistency, these spots have been called "yellow-blotch local lesions." If the virus used for inoculation was a concentrated sample, the yellow-blotch local lesions are numerous, and quickly merge into one another and become indistinguishable. If the virus was more dilute, however, the local lesions are more scattered, and they can be observed to increase in size gradually from 2 or 3 mm. to about 5 or 6 mm. in diameter during a period of three or four days. By this time systemic symptoms have become visible as marked "clearing of the veins" on the youngest leaves.

It is when the leaves are killed in steam, decolorised in alcohol, and stained in iodine, however (Holmes 1931 for tobacco mosaic; Bewley 1930 for a yellow tomato mosaic), that the effect of the virus becomes particularly evident and striking. (The preparations are naturally made

towards the end of the day, when starch formation has proceeded to a maximum in the normal parts of the leaf. The stained leaves can be floated out on paraffined paper, and then placed between paraffined paper and blotting paper and pressed overnight. Leaves dried in this way are convenient for storing in herbarium envelopes.) Yellow tobacco mosaic has such a severe effect on the chloroplasts that starch formation is inhibited entirely in the areas of the yellow-blotch local lesions (Plate XXXIV, figs. 2, 4), and in the areas of the young leaves where clearing of the veins becomes evident on systemic invasion (Plate XXXIV, figs. 5, 6). After the staining in iodine the affected areas show as clear spots where the leaf has been unable to form starch, and by making preparations on successive days the development of the local lesions on the inoculated leaves can be followed from a stage before they are visible to the naked eye, through successively later stages in their spread.

Although to the naked eye there is never anything more visible than a faint yellowish blotch, the iodine treatment reveals the path of travel of the virus in a most illuminating way. The enlargement of the local lesion is seen to be circular for only a very short time, and it soon develops angular points owing to the more rapid travel of the virus along any veins which are met. Successive stages of the spread of the virus in the primary lesion are shown in Plate XXXV, and it will be seen how slow is the movement of the virus across the lamina of the leaf in comparison with its movement down any adjacent larger vein leading to the petiole of the leaf.

The virus apparently gets into the veins and is carried up to the top of the plant, causing the appearance of systemic symptoms, while the primary lesion on the inoculated leaf is still comparatively restricted in size (about the stage shown in Plate XXXV, fig. 2, for example). The showing up of the track of the virus down the leaf (Plate XXXIV, fig. 7, and Plate XXXV, figs. 5–8), which becomes visible later, would thus seem to result from the slow outward movement of the virus from the vein it travelled down, affecting the chloroplasts of the neighbouring cells. Even at a comparatively late stage, when systemic symptoms have been visible for days, no virus may be detectable in the unaffected parts of the lamina of the inoculated leaf (such as *A*, Plate XXXV, fig. 7), while abundant virus is present in the gradually enlarging primary lesion (*B*, same figure).

The dark border round the areas affected by the virus, as seen in Plate XXXIV, fig. 4, and in Plate XXXV, figs. 6–8, is due to more starch being present in the cells there than is in the cells of the

unaffected portions of the lamina of the leaf. This would suggest that the initial effect of the virus on the cells may be one of stimulation. It may possibly be due, however, to temporary inhibition of translocation. It should not be difficult to decide by experiment between these two possibilities.

The above work was carried out while the writer was enjoying an Honorary Fellowship in Plant Pathology, awarded him by the Regents of the University of Wisconsin.

SUMMARY.

It is shown by means of dilution tests that a light rubbing with virus, in which no visible wound is produced on the leaf, is a more effective method of mechanical inoculation than scratching with a needle in the case of the five viruses: cucumber mosaic, spot necrosis, tobacco ring spot, petunia mosaic, and yellow tobacco mosaic, all inoculated on to tobacco. The contentions of Holmes with regard to inoculating methods with tobacco mosaic are thus extended to these other viruses.

Some of these viruses form local lesions of a definite type which may prove to be of value in quantitative work. It is shown that temperature, age of the leaf inoculated, and other factors influence the character and the number of the local lesions formed.

The development of the virus in the inoculated leaf (the "primary phase" before systemic symptoms become visible), as well as certain later stages, can be followed particularly well in the case of yellow tobacco mosaic, using the iodine method for demonstrating the areas of normal starch formation (Holmes, 1931). In an area closely corresponding to where the virus has spread, starch formation is inhibited, so that the path followed by the virus shows up as a light-coloured area in the treated leaves. An intimate relation between the vascular system and the path of travel of the virus is made strikingly evident by this treatment.

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EXPLANATION OF PLATES XXXIV—XXXVI.

PLATE XXXIV.

Fig. 1. Illustrating the method of inoculation by rubbing the surface of the leaf with a sterile glass spatula dipped in virus, the leaf being supported by the fingers which are protected from contamination by a square of clean paraffin paper. The under sides of the glass spatulas are ground flat on a carborundum wheel.

Fig. 2. Tobacco leaf which has been inoculated on the left side with five needle scratches, and on the right side by rubbing with the virus of yellow tobacco mosaic diluted 1 : 10,000. Three days after inoculation the leaf was removed from the plant and treated by the iodine method, which shows up the points of entry of the virus. Note how the only two successful points of entry of the virus along the needle scratches were in this case at the very ends of the scratches, where the pressure would have been lightest.

Fig. 3. Tobacco leaf three days after inoculation by rubbing with the virus of yellow tobacco mosaic diluted 1 : 10,000. Very faint yellow-blotch local lesions are visible in places.

Fig. 4. The same leaf the same day after killing, decolorising in alcohol, and treatment with iodine. The points of entry of the virus, which were visible on the fresh leaf as very faint yellow-blotch local lesions or not visible at all (Fig. 3), now show up very clearly owing to no starch being formed at these points. Note the darker borders round the spots, where more starch is present than in the normal parts of the leaf.

Figs. 5, 6. Two iodine-treated leaves from the centres of plants inoculated six days previously with yellow tobacco mosaic. When on the plant these leaves showed the "clearing of the veins" characteristic of early systemic invasion. After treatment by the iodine method the areas invaded by the virus show up very clearly. Tests from the tips of such leaves show no virus to be present much beyond the cleared areas. A variety of patterns is produced on these young leaves, depending probably on the translocation movements within the leaf when the virus entered, as influenced by age of leaf, time of day, temperature, etc.

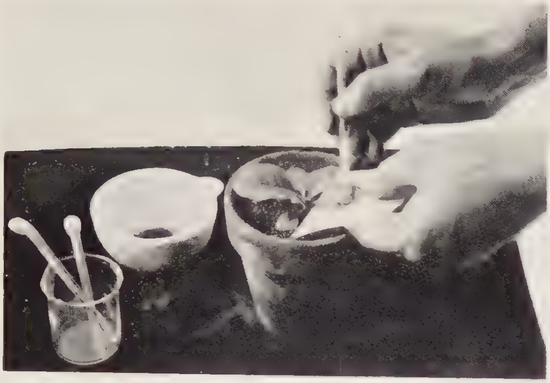
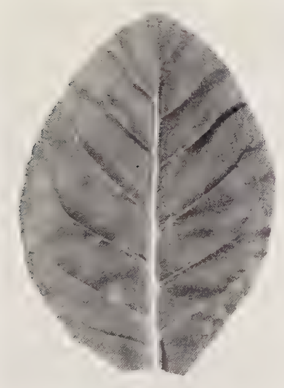


Fig. 1.

Fig. 2.



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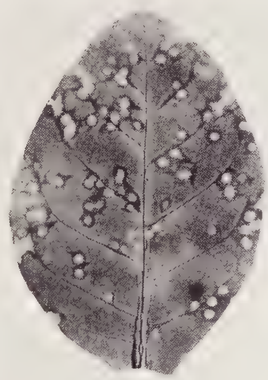


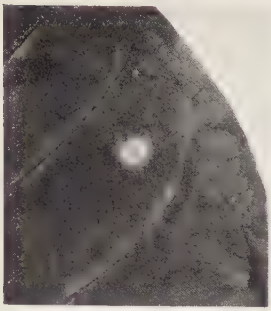
Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

SAMUEL.—Some Experiments on Inoculating Methods with Plant Viruses, and on Local Lesions (pp. 494–507).



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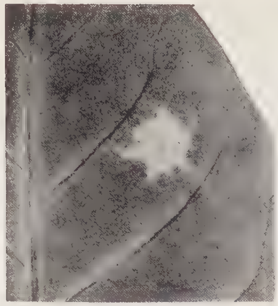

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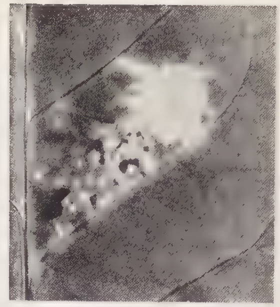

Fig. 3.

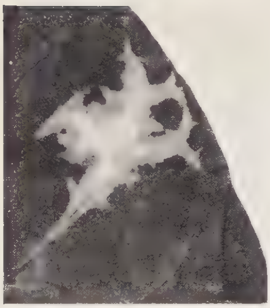

Fig. 4.

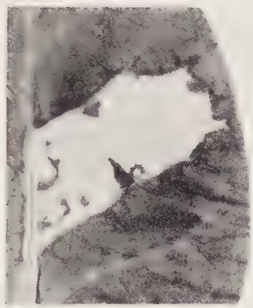

Fig. 5.

Fig. 6.

Fig. 7.

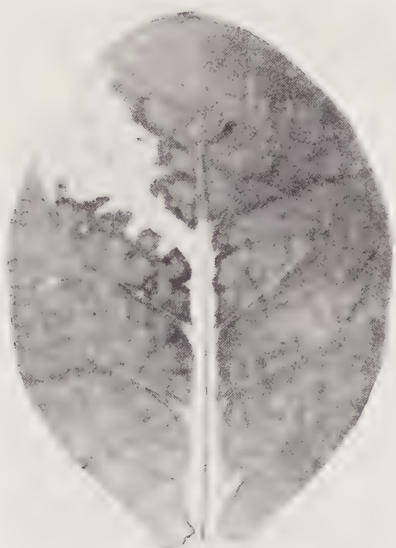

Fig. 8.

SAMUEL.—Some Experiments on Inoculating Methods with Plant Viruses, and on Local Lesions (pp. 494–507).

Fig. 1.

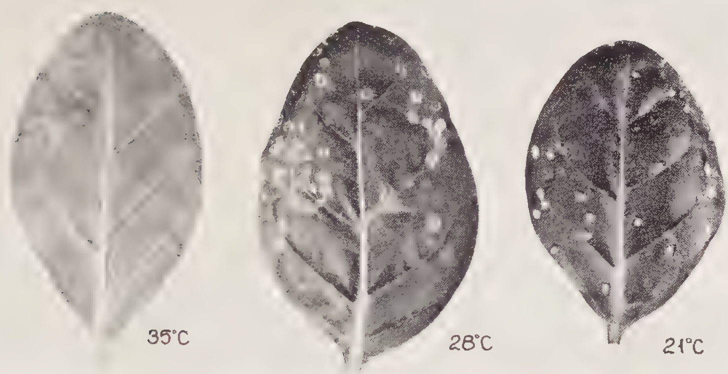

Fig. 2.

Fig. 3.

SAMUEL.—SOME EXPERIMENTS ON INOCULATING METHODS WITH PLANT VIRUSES, AND ON LOCAL LESIONS (pp. 494–507).

Fig. 7. Iodine-treated tobacco leaf which was wiped with a very dilute yellow tobacco mosaic virus so that only one local lesion resulted. Treated eight days after inoculation. Before the leaf was pressed the track of the virus was visible right down one side of the main vein to the petiole.

PLATE XXXV.

All the figures on this plate are of tobacco leaves which were inoculated with yellow tobacco mosaic at one point by touching with a glass rod dipped in virus, the leaves being later treated by the iodine method at various intervals after inoculation. All plants were grown in a greenhouse kept at a temperature of approximately 28° C.

Figs. 1–3. Stages in development of local lesions when the inoculum was applied at a point between two lateral veins. Three, five, and ten days after inoculation respectively.

Fig. 4. When the inoculum was applied directly over a lateral vein; nine days after inoculation.

Figs. 5, 6. Later stages on a slightly smaller scale. Fig. 5, when the inoculum was applied between two veins; Fig. 6, when the inoculum was applied directly over a vein. Twelve days after inoculation.

Figs. 7, 8. Later stages showing the path of the virus down the midrib. Fig. 7, when the inoculum was applied between two veins; Fig. 8, when the inoculum was applied directly over a vein. Fourteen days after inoculation.

PLATE XXXVI.

Effect of Temperature on Local Lesions.

Fig. 1. Tobacco mosaic on *N. glutinosa.* Leaves from plants which, after wiping with virus, were kept at temperatures of 35° C., 28° C. and 21° C. Photographed three days after inoculation. Faint yellowish local lesions were present on the leaves of the plants kept at 35° C.

Fig. 2. Spot necrosis on *N. rustica.* Leaves from plants which, after wiping with virus, were kept at temperatures of 35° C., 28° C. and 21° C. Photographed five days after inoculation. No local lesions were formed on the leaves of the plants kept at 35° C.

Fig. 3. Spot necrosis on *N. acuminata.* Leaves from plants which, after wiping with virus, were kept at temperatures of 35° C., 28° C. and 21° C. Photographed five days after inoculation. No local lesions were formed on the leaves of the plants kept at 35° C.

(*Received March 9th*, 1931.)

THE SUSCEPTIBILITY OF CERTAIN POTATO VARIETIES TO LEAF-ROLL AND MOSAIC INFECTION

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(With Plate XXXVII.)

Introduction.

In a previous paper (1) one of the writers described field trials to determine the susceptibility of some twenty-three varieties to leaf-roll and mosaic infection. Several varieties were discarded for one reason or another and ultimately complete results were obtained from only ten of them. It was shown that "susceptibility" to leaf-roll could be expressed in three ways, viz. as the liability of the haulm of the "parent" plant to contract infection, the liability of the tubers produced by such a plant to contract infection, and finally, the effect on the yield when infected tubers were planted. Great variation existed in these forms of susceptibility in the same variety and different varieties reacted quite differently in many cases. The variety Crusader, for instance, showed all three forms of susceptibility in an extreme manner; Up-to-Date was very susceptible to infection both of haulm and tubers but the virus had little effect on the yield; on the other hand, the variety Great Scot exhibited considerably less susceptibility of haulm and tubers to infection, as compared with Up-to-Date, but appreciably more effect was produced on the yield. The opinion was expressed that only by such field trials could one estimate the probable effect of the introduction of new varieties into localities where virus diseases exist and spread rapidly.

Attempts are being made by some workers to build up stocks of certain varieties tested under glasshouse conditions for freedom from virus diseases. This is a Herculean task complicated by both purely scientific and practical difficulties. On the scientific side, research is revealing an increasing number of diseases carried without symptoms or appreciable loss of vigour. The number, sources, and vectors of these diseases being quite unknown, there is no real reason to believe that

potato viruses are even restricted to the Solanaceae. It would be unwise, therefore, to expect virus-free stocks to retain their health in the field—even under relatively isolated conditions. Even if the scientific difficulties were overcome, the impracticability of maintaining sufficient healthy stocks to plant the national acreage under potatoes, and the necessity for a complete clearance of all present commercial stocks, would remain. In the writers' opinion, the practical problem for a number of years to come is to eliminate all varieties which are seriously affected in yield by infection with virus diseases, and to concentrate on those which are "tolerant."

New trials of susceptibility were begun in 1929 with this as the primary object, but with the hope also that some progress might be made in deciding the most practical way of conducting such trials on a large scale in the future. Seven varieties were included of which two had previously been tested in 1924 (1), so that the influence of "season" could be noted and some idea obtained of the degree of general application of results to other seasons or localities.

"LAY-OUT" OF TRIAL IN 1929.

The previous work (1) had shown the need for a proper selection of the disease infectors. The mosaic infectors of the variety Irish Chieftain, on that occasion, produced very stunted plants of the "curly-dwarf" type, and the transmission of mosaic was very irregular. In the present work, however, a stock of Golden Wonder with mosaic was used as the source of infection; this variety being selected partly because symptoms other than mosaic are rarely or never seen under field conditions. The leaf-roll infectors were from a stock of Arran Comrade which has shown the disease for many years and is maintained solely for use as infectors as occasion arises.

The most obvious difficulty in all such field trials is to secure uniform chances of infection, which in practice means to eliminate the risk of a haphazard distribution of the aphis vectors. The plan adopted to secure this end was to plant four replications of single drills of each variety. Each of the four sets of the seven varieties constituted a plot with the varieties randomised so that each occupied a different position, with different neighbours, in the various plots. Text-fig. 1 shows the plan of the trial concerned with leaf-roll infection, the mosaic plots were similar and formed a continuation of the same drills. Diseased infectors were planted in drills alternating with those of the varieties under test, leaf-roll plants being used in Plots 1–4, and mosaic in Plots 5–8.

It was necessary to ensure that only normal, *i.e.* symptomless, plants were planted. This was done by halving each tuber and planting corresponding halves in the same relative position in the leaf-roll and mosaic plots, so that any given plant in the leaf-roll section had its easily recognised counterpart, grown from the same tuber, in the mosaic section. Similar symptoms in the two half-tubers were considered to be proof of prior infection of the tuber, which could then be eliminated from the trial. In point of fact no case of leaf-roll and only one of definite mosaic occurred, although a faint mottling was present on most plants of the variety Kerr's Pink, but as that appears to be the normal condition of stocks of this variety it was retained for the purposes of this trial.

Prevailing wind

Plot 1	Plot 2	Plot 3	Plot 4
Field Marshal	Great Scot	Herald	Arran Crest
Arran Crest	Kerr's Pink	Arran Consul	Arran Banner
Arran Banner	Field Marshal	Great Scot	Herald
Arran Consul	Arran Crest	Arran Banner	Arran Consul
Great Scot	Herald	Kerr's Pink	Field Marshal
Herald	Arran Banner	Arran Crest	Kerr's Pink
Kerr's Pink	Arran Consul	Field Marshal	Great Scot

Text-fig. 1. Plan of field trial (leaf-roll) in 1929. -------- = drills of infectors.

The prevailing direction of the wind was oblique to the drills, so that no wholesale transmission of the one virus disease to the other section was anticipated. On the other hand, a little admixture of viruses was expected to occur by the juxtaposition of the leaf-roll and mosaic sections. This was regarded as an advantage in that the behaviour of the one virus in presence of the other, as well as separate infections, could then be studied.

The unit drill consisted of twenty plants grown from half-tubers as already described, so that in all eighty plants of each variety were exposed to leaf-roll infection and a similar number to mosaic infection.

A. *Results of planting out the progeny in* 1930.

In 1930 the whole progeny of every experimental plant—some 12,000 tubers in all—were graded into ware, seed and chats, and planted in order to determine the degree of infection which had occurred the previous year. Since it was necessary to be able to identify each plant

produced, the tubers were dibbled in 15 in. apart with 30 in. spaces separating the progeny of different "parent" plants. Notes were made of the size and condition, *i.e.* "hardness" and occurrence of secondary growth, of each tuber when planted.

The difference in the effect of mosaic and leaf-roll respectively on all the varieties was very pronounced from the first appearance of the plants above ground and throughout the season. The progeny of the plants exposed in 1929 to mosaic infection came above ground much more quickly and remained far more vigorous than the progeny of the corresponding half-tubers which had been exposed to leaf-roll infection, a fact which is clearly brought out by Plate XXXVII, fig. 1. With the exception of the variety Great Scot in which traces of mottling could be seen in only a few plants, there was ample evidence that mosaic, in the form of a slight mottling, had been transmitted generally. The limitations of field trials for the determination of transmission of such faint symptoms were, however, as evident in this work as in that previously undertaken (1). Little more, indeed, could be done than to note the very slight effect the mottle had produced on the vigour of these stocks as compared with normal stocks growing near at hand. The variety Kerr's Pink, which in the previous year had shown similar faint mottling, now exhibited it in a rather more intense form, but not so as to reduce the vigour to any appreciable extent.

B. *Percentage plants contracting leaf-roll in* 1929.

The occurrence of a single case of secondary leaf-roll in the progeny of a plant in 1930 was considered to be sufficient evidence that the parent plant had contracted the disease in the previous year; the likelihood of such symptoms being due to current year infection, as has elsewhere been shown by the writers to be possible (2), is in this case negligible, since the plants were well past the critical stage of growth before the onset of aphis vectors. On this basis practically all the plants in every variety had received the virus in 1929, the actual percentage infection being Arran Crest 96·9, Herald 98·4, Great Scot 100, Arran Consul 100, Arran Banner 96, Kerr's Pink 100, Field Marshal 100.

This is in marked contrast to the range of plant infection (*i.e.* 100–60 per cent.) occurring in the 1924 trial when aphides were much less abundant than in 1929. On the contrary the aphis infestation in 1929 was evidently sufficient to give uniform chances of infection to all varieties, and the result shows that under heavy conditions of infestation no differences of susceptibility of haulm may be apparent. Such a

marked dependence upon seasonal factors, influencing the breeding of aphides, obviously militates against the usefulness of field trials if the susceptibility of a variety is to be judged by the liability of the haulm to contract the disease.

C. *Percentage progeny contracting leaf-roll in* 1929.

Here we appear to be on safer ground in the estimation of susceptibility to leaf-roll, for the percentage of infected tubers produced by these infected plants varied considerably; these being, Arran Crest 77·3, Herald 90·4, Great Scot 90·9, Arran Consul 99·9, Arran Banner 86·8, Kerr's Pink 99·0, Field Marshal 23·7. Using this as a standard of susceptibility, Field Marshal, Arran Crest and Arran Banner appear to be more resistant, in the sense that a higher proportion of the tubers escaped infection, than the other varieties. The influence of season is again apparent, for although the varieties Great Scot and Kerr's Pink occupy the same relative positions as in 1924, the tuber infection in that year was much lower, *i.e.* 65·5 and 78·1 per cent. respectively. It may be assumed, therefore, that not only was infection less heavy in 1924, but also occurred later in the season than was the case in 1929. It is believed that the proportion of tubers contracting infection from the haulm bears a close relation to the date of haulm infection, that is, to time in relation to tuber formation. It follows that when aphis infestation is exceptionally heavy, or early, any real differences in the liability of the tubers to contract disease may be obscured.

An even more striking difference was the degree of stunting and the proportion of leaves actually rolled in the infected progeny in 1930. Although the varieties Herald, Great Scot, Arran Consul and Arran Banner, showed approximately similar numbers of infected plants, the first and third varieties were very badly rolled and stunted, and it was obvious that the yield would be very small. On the other hand, the majority of the infected plants of the varieties Great Scot and Arran Banner had only the lowest leaves rolled and the plants had apparently suffered little in loss of vigour. A separate note was, therefore, taken of the number of plants of these two varieties in which the rolling was sufficiently severe to affect the vigour and thus the probable cropping power. On this basis only 10·8 and 20·7 per cent. of the diseased Great Scot and Arran Banner, respectively, were seriously affected (cf. Plate XXXVII, fig. 2).

D. *Effect of leaf-roll and mosaic on yield.*

Although the liability of haulm, and still more so of tubers, to contract infection is an important criterion of susceptibility from the point of view of the seed producer, the effect of the diseases on the yield is of paramount importance to the ordinary grower.

Table I gives the mean yield, in numbers and weight of tubers, obtained from four lots of twenty normal plants growing close to the infected plots in 1930, and from a similar number of diseased plants from the leaf-roll and mosaic plots respectively. No selection of plants was made other than to ensure that in all cases the plants had been grown from similar seed sets.

Table I.

Mean yields, in lb. from normal, leaf-roll and mosaic plots.

	Normal		Leaf-roll				Mosaic			
Variety	No.	Weight	No.	% loss	Weight	% loss	No.	% loss	Weight	% loss
Arran Crest	119	32·9	29	75·6	1·50	95·2	99	16·8	27·4	16·6
Herald	204	43·75	69	66·2	1·05	97·6	224	− 9·8	33·1	24·4
Great Scot	133	37·6	114	14·3	19·00	49·5	185	−39·1	42·6	−13·2
Arran Consul	187	46·2	65	65·3	6·31	86·4	169	9·7	42·2	8·6
Arran Banner	140	46·3	98	30·0	17·81	61·6	152	− 8·6	50·9	−10·0
Kerr's Pink	195	45·56	101	48·2	13·25	70·9	210	− 7·7	39·4	13·4
Field Marshal	170	43·9	145	14·7	32·50	26·0	214	−25·9	43·6	0·8

One cannot expect precision from such a small trial, but there is sufficient evidence to divide the varieties into two groups so far as leaf-roll is concerned, *i.e.* Field Marshal, Great Scot and Arran Banner, being definitely tolerant and the remainder intolerant of the disease. Even so, there is a surprisingly heavy loss from the Great Scot and Arran Banner in view of the fact that only 10·8 and 20·7 per cent. respectively of the infected plants of these varieties had aroused any apprehensions of a serious fall in yield. Evidently, the degree of rolling of the foliage shown by an infected plant is not a reliable guide to the probable loss in yield.

The loss in yield due to mosaic infection is also larger than was anticipated from the vigour of the plants during the season. It is abundantly clear, however, that mosaic alone is far less serious in its effects on yield than is leaf-roll (cf. Plate XXXVII, fig. 1), though it will be shown later that when combined with leaf-roll, mosaic may prove extremely serious.

The fact that in both diseases the loss in weight of tubers is in all cases greater than the loss in numbers of tubers points to a reduction

in size as a consequence of infection. This is of importance to the commercial grower, who is less concerned with loss in total yield than with loss in the saleable crop, *i.e.* ware and seed. Table II gives the proportion of ware plus seed to total crop in normal, leaf-roll and mosaic plants, from which it will be seen that whereas mosaic has had little effect leaf-roll, on the contrary, has in some cases seriously reduced the proportion of saleable crop. In the variety Herald, for instance, the almost negligible crop from leaf-roll plants is itself almost entirely composed of chats, useless except as pig food.

Table II.

Percentage ware plus seed in total crop.

Variety	Normal	Leaf-roll	Mosaic	Crinkle
Arran Crest	96	59	97	—
Herald	94	19	92	—
Great Scot	97	93	96	—
Arran Consul	97	85	96	—
Arran Banner	98	95	98	—
Kerr's Pink	95	87	94	—
Field Marshal	96	95	95	93

The three varieties Field Marshal, Arran Banner and Great Scot again fall into a class apart from the remainder in the relatively small effect on the size of the tubers produced by leaf-roll.

E. *The effect of one virus in the presence of another.*

As has already been stated the mosaic section of the trial in 1929 was planted in a continuation of the same drills occupied by the leaf-roll section. The prevailing direction of the wind was diagonally across the plots, so that there was more likelihood of mosaic transmission to the leaf-roll plots than of leaf-roll transmission to the mosaic plots. This was found to have been the case when the progeny were planted in 1930, since a very large proportion of the progeny of the four leaf-roll plots developed a mottle, although the number showing definite mosaic was small. The transmission of leaf-roll was much less in amount and its spread through the four mosaic plots is indicated in Table III.

The chances of leaf-roll infection in Plots 1–4 were extremely high—far more so than would normally occur in any ordinary field crop. An approximation to ordinary field conditions, in the degree of exposure to infection, is, however, to be found in Plots 5–8, and it is of considerable interest to find that Great Scot, Arran Banner and Field Marshal again show a higher degree of resistance to infection than the other varieties. The reason for the occurrence of disease in the larger

tubers only of such plants as were only partially infected, is not understood, but further work is in progress in an effort to find some explanation.

Table III.

Spread of leaf-roll through the four "mosaic" plots in 1929.
(*Twenty plants of each variety in each plot.*)

Variety	Plot 5	Plot 6	Plot 7	Plot 8
Arran Crest	9 plants, all tubers	2 plants, all tubers	1 plant, 1 ware only	2 plants, 1 seed and 2 seed
Herald	5 plants, all tubers	No infection	No infection	No infection
Great Scot	1 plant, ware only	No infection	No infection	No infection
Arran Consul	2 plants, most tubers	No infection	No infection	No infection
Arran Banner	No infection	1 plant, ware only	No infection	No infection
Kerr's Pink	3 plants, all tubers	No infection	No infection	No infection
Field Marshal	3 plants, 1 ware on each plant	No infection	No infection	No infection

In all the varieties except Field Marshal the effect of superimposing one virus upon another in the same plant was simply to produce both sets of symptoms, which could be diagnosed without much difficulty. In Field Marshal, however, a large number of plants showed neither typical leaf-roll nor mosaic, but the necrotic, downward curling leaves associated with the disease "crinkle." The distribution of the crinkle symptoms throughout all eight plots is given in Table IV, and, in the writers' judgment, leaves no doubt that they are the result of mixed leaf-roll and mosaic infection.

The close morphological similarity between Field Marshal and the variety Up-to-Date, and the reported invariable occurrence in the latter variety of a "streak" virus carried without symptoms (3), opens up the question as to whether the "crinkle" found in the variety Field Marshal is not also due in part to the action of a similar hidden streak in this variety. This cannot be answered without critical glasshouse work, but the fact that the crinkle did not occur in all the progeny scarcely suggests the influence of such a systemic streak virus already present in the parent plant. Nor is its presence suggested by the fact that the proportion of tubers affected by crinkle diminishes with the chances of mixed leaf-roll and mosaic infection.

The distribution of typical leaf-roll symptoms in the four "leaf-roll" plots of this variety is also of interest, these being Plot 1, 32·0 per cent.;

Plot 2, 31·6 per cent.; Plot 3, 12·5 per cent.; Plot 4, 18·7 per cent. In some cases leaf-roll and crinkle symptoms appeared in different haulms of the same plant, but in the main, leaf-roll occurred without crinkle symptoms only in the plants derived from chats, or more rarely, from seed sets; no cases of simple leaf-roll were found in plants derived from ware sets (cf. Plate XXXVII, fig. 3). From this it would appear that the crinkle symptoms are in some way affected by the relative proportions of the two viruses, leaf-roll and mosaic, entering the tuber, but the whole problem is obscure and requires critical glasshouse work for its elucidation.

Table IV.

Incidence of "crinkle" in the four leaf-roll and four mosaic plots of Field Marshal.

Leaf-roll				Mosaic			
Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8
18/19 plants, 158 out of 204 tubers or 77 %	20/20 plants, 142 out of 180 tubers or 79 %	16/16 plants, 154 out of 168 tubers or 91 %	19/19 plants, 189 out of 229 tubers or 82 %	4/20 plants, most tubers	1/20 plants, one tuber	1/20 plants, six tubers	1/20 plants, one tuber

It has already been pointed out that the variety Field Marshal suffered relatively little from leaf-roll alone. This is not the case, however, when the crop from plants affected with crinkle is compared with that from normal plants. Four lots of twenty normal plants produced a mean number of 170 tubers weighing 43·9 lb., whereas a similar number of crinkle plants gave only 70 tubers weighing 10·25 lb., a loss of 58·8 per cent. in numbers and 76·7 per cent. in weight. On the other hand, 93 per cent. of the crop from the crinkle plants were graded into the ware and seed class as against 96 per cent. from the normal plants. It appears, therefore, that the effect of crinkle is mainly restricted to reducing the number rather than the size of tubers produced by an infected plant.

Discussion.

The justification for carrying out elaborate field trials to determine the susceptibility of potato varieties to virus disease infection rests upon the following facts:

1. The difficulties in the way of producing, and maintaining in the field, perfectly healthy stocks of potatoes, are very great. Even if they are overcome, many years must elapse before commercial stocks can be replaced by virus-free material. In the interim some test of the reaction of popular, and new, varieties to infection must be made.

2. Glasshouse tests involving the use of grafting methods or of insects under controlled conditions cannot imitate these field conditions under which the variety must eventually compete with others, particularly as the ultimate criterion of the value of a variety is its yield, and this cannot be tested under glass.

3. No satisfactory method of diagnosing virus diseases in the unplanted tuber is known. A promising method based on the fact that diseased tubers lose water more slowly than do healthy ones, and hence retain their hardness, was discovered by McLean (4). The opportunity was taken by the writers to test the value of this laboratory method, in the field, when carrying out the present trial. A note was taken of the degree of "hardness" shown by some 12,000 tubers when handled at planting time, but it was found impossible to forecast the health of the plants grown from these tubers in this way. Since this appears to be the only practicable way in which hardness could be determined on a large scale by growers, the method obviously cannot replace proper field trials involving the planting of the progeny of plants exposed to infection.

It would appear that replication and randomising of varieties are essential to secure uniform chances of infection, but, in addition, there must be an abundance of infector plants. The chief objection to this is that when aphides are unusually common the virus infection produced will be much heavier than would occur with a similar abundance of aphides under ordinary field conditions and quite real differences in susceptibility might be obliterated. With this in mind, an improvement in the plan adopted in the present work could be effected by alternating the plots containing the diseased infectors with other plots similar except that infectors would be excluded, so that useful results might be expected whether aphis infestation were heavy or light. Indeed, there can be little objection to alternating "leaf-roll" plots with "mosaic" plots instead of with "no infector" plots, since, after all, what is wanted is the reaction of the variety to mixed virus infection. Had mosaic been excluded from the present trials an altogether false impression would have been given of the reaction of Field Marshal under ordinary field conditions.

Assuming equal chances of infection to be secured by replication and randomising the varieties, the percentage haulm infection in that year, as revealed by the occurrence of even a single case of infection in the progeny, will give some indication of differences in susceptibility providing aphis infestation is not very heavy. The percentage progeny contracting disease from the infected haulm shows the same dependence upon seasonal factors which affect the breeding of aphides. When aphis

infestation is not heavy the difference in the number of tubers so contracting disease forms a useful guide to the susceptibility of the variety.

The effect on the yield is, however, the best criterion of susceptibility so far discovered. This effect cannot be estimated by the degree of stunting produced nor by the number of rolled leaves on a plant, but necessitates the weighing and grading of crops from diseased and normal plants growing as far as possible under similar conditions. The present trial showed that the actual loss in varieties affected with mosaic, and in those which remained vigorous when infected with leaf-roll, was much greater than was anticipated.

It is doubtful how far the results of a single trial of the effects produced by these diseases can be applied to other seasons or localities and more field trials are required before a definite opinion is expressed. It is at least justifiable, however, to draw attention to a comparison of the results obtained in 1924 and in 1929, as given in Table V.

Table V.

Loss in cropping power due to leaf-roll in 1924 *and* 1929.

Variety	% loss by weight	% loss numbers
Herald	97·6	66·2
Arran Crest	95·2	75·6
Crusader	95·0*	84·0*
Arran Consul	86·4	65·3
Kerr's Pink	70·9 (65·0*)	48·2 (54·0*)
Majestic	65·0*	53·0*
Tinwald Perfection	63·0*	53·0*
Arran Banner	61·6	30·0
Ben Cruachen	60·0*	60·0*
Arran Comrade	51·0*	33·0*
Great Scot	49·5 (45·0*)	14·3 (29·9*)
Ben Lomond	47·0*	33·0*
Katie Glover	47·0*	41·0*
Field Marshal	26·0	14·7
Up-to-Date	14·0*	40·0*

* = 1924 trial.

The varieties in the table are given in descending order of their susceptibility, and it is evident that the loss in Kerr's Pink and Great Scot is of the same order in both trials. It will be remembered that these varieties, in common with others, showed a much heavier haulm and tuber infection in 1929 than in 1924. This, however, has scarcely affected the percentage loss by weight, although the tubers produced were somewhat smaller in 1929 than in 1924. If it is true that seasonal factors have little effect on the extent to which the crop is reduced when the plants are infected with leaf-roll, we may take Table V as giving approximately a scale of susceptibility. It is in any case fairly safe to assume

that varieties near the top of the scale will usually suffer much more as a result of leaf-roll infection than will those near the bottom of the table.

SUMMARY.

1. A trial planned to ascertain whether any differences in susceptibility to leaf-roll and mosaic infection existed in seven varieties—two of which had been included in a previous trial—was laid down in 1929. It consisted of four replicated plots for each disease; the varieties being randomised in the plots to secure uniform chances of infection. Sources of infection were provided by drills of diseased plants alternating with varieties under test.

2. Mosaic was generally transmitted but with such faint symptoms that percentage infection could not be determined. The apparent loss of vigour was very slight, but losses ascertained by weighing the crop, against that given by normal plants, ranged from 0·8 to 24·4 per cent.

3. Leaf-roll infection was very heavy; most varieties showing from 90 to 100 per cent. infection of the plants. Tuber infection on the infected plants was also heavier than in 1924, and ranged from 77·3 to 99·9 per cent. Both haulm (or plant) infection and tuber infection thus showed a marked dependence upon seasonal factors which affect the breeding of insect vectors.

4. The loss in yield due to leaf-roll was found to be the most reliable guide to the susceptibility of a variety. This loss cannot be estimated from the degree of stunting or the number of rolled leaves on a plant, but only by weighing and grading the crop. Some evidence is presented to show that loss in yield of an infected plant is less dependent upon seasonal factors than percentage haulm or tuber infection, and that, therefore, figures showing such losses have a more general applicability to other localities and seasons. On this assumption a table of susceptibility of fifteen varieties has been constructed.

5. Although the variety Field Marshal suffered little from leaf-roll infection, heavy losses occurred when mixed leaf-roll and mosaic viruses entered the plant. In large tubers receiving mixed infection the effect was to produce neither leaf-roll nor mosaic symptoms, but "crinkle." The small tubers, however, gave apparently pure leaf-roll.

6. The value to be attached to field trials of this kind, and their applicability to other conditions, is discussed in detail. Suggestions are offered for improvement in the technique for such trials.

The writers gladly acknowledge the help given by the laboratory attendant, Mr G. L. Turner, in carrying out this work, and also the support given by Prof. R. G. White in extending ample facilities on the College Farm.

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EXPLANATION OF PLATE XXXVII.

Fig. 1. General view of appearance of progeny of plants exposed to leaf-roll or mosaic in 1929. Leaf-roll on left, mosaic on right. A = Arran Banner, B = Arran Consul, C = Great Scot, D = Herald, E = Arran Crest, F = Field Marshal exposed to mosaic.

Fig. 2. Effect of leaf-roll on vigour. Great Scot (A) and Arran Consul (B).

Fig. 3. Effect of mixed leaf-roll and mosaic on Field Marshal. (A) and (B) = crinkle in ware and seed respectively. (C) = leaf-roll alone showing in plant derived from a chat.

(*Received February* 12*th*, 1931.)

Fig. 1.

Fig. 2.

Fig. 3.

WHITEHEAD AND CURRIE.—THE SUSCEPTIBILITY OF CERTAIN POTATO VARIETIES TO LEAF-ROLL AND MOSAIC INFECTION (pp. 508–520).

SPRAIN OR INTERNAL RUST SPOT OF POTATO (*B. RUBEFACIENS*)

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(With Plate XXXVIII.)

In a previous investigation of "Sprain or Internal Rust Spot of Potato" (1) the attempt to reisolate *Bacterium rubefaciens* from the artificially infected tubers was unsuccessful. In view, however, of the difficulty previously experienced of culturing this elusive organism the failure to reisolate it from a limited supply of material was not very surprising.

The symptoms of the disease as reproduced were so convincing that, in spite of the non-compliance of the work with Koch's third postulate for pathogenicity, there was little room for doubt that *B. rubefaciens* was the causal organism. The results of the investigation were therefore published. Since that time the work has been repeated and brought to a satisfactory conclusion. In view of the delicacy of the technique involved, the data of the experiment to be described are given in full detail.

Method of conducting the experiment.

Soil was procured from a field where the disease was known to occur and introduced into twelve 10-inch flower pots, the whole being autoclaved at 15 lb. pressure for two hours. After an interval of twenty days six of the pots were inoculated with an emulsion of *B. rubefaciens*, the remaining six pots being left as controls. New seed tubers from Scotland of the varieties Golden Wonder and Field Marshal, which proved on cutting to be free from the disease, were chosen for the experiment. These were sterilised by immersion in an 0·1 per cent. solution of mercuric chloride for thirty minutes. Two tubers of Field Marshal and four of Golden Wonder were cut into halves, the cut surfaces being allowed to suberise. One half of each tuber was planted in an inoculated pot and the other half in a control pot. At intervals of a fortnight throughout the growing season the inoculated pots were given an emulsion of *B. rubefaciens* which was poured down sterilised glass tubing previously inserted to a depth of about 2 inches into the soil. Since *B. rubefaciens*

gives such slow and comparatively weak growth in culture, the soil inoculation so obtained was by no means excessive. When the plants had died down the pots were stored in a cool place and kept slightly damp until lifted.

Examination of tubers.

The tubers were lifted during the last week of January, 1930, and cut into very thin slices.

The tubers from the control pots were all free from the least trace of the disease. The results from the inoculated pots were as follows:

No. of pot	Variety	No. of tubers in crop	Results of inoculation
1	Field Marshal	6	One tuber positive. A very small spot in the medulla which proved on sectioning to be a definite "Sprain" lesion
2	„	7	Two tubers positive. In one tuber there was a small brown lesion in the cortex. In the other a general infection consisting of small lesions both in the cortex and medulla
3	Golden Wonder	6	One tuber positive, showing a small brown lesion in the cortex
4	„	8	Two tubers positive. In one of these large lesions were shown in the medulla extending from the heel end half way through the tuber (see Plate XXXVIII, fig. 1)
5	„	4	All negative
6	„	6	Four tubers positive, showing small spots in the cortex and medulla (see Plate XXXVIII, fig. 2). Each affected tuber showed three or more spots which, on sectioning, proved to be typical "Sprain" lesions

It is noteworthy that the larger lesions produced in these inoculations occurred in the medulla and in all respects resembled those found in the pith of naturally affected tubers (cf. (1), p. 577).

Re-isolation of the organism.

The media used were "Soil Extract Solution" and "Soil Extract Agar" made with soil from the same source as that used in the experiment.

The tubers before being cut were washed in three changes of water, immersed in absolute alcohol for a few seconds, and dried with a sterile towel. They were then sliced with a sterile scalpel with every precaution against contamination. On the discovery of a lesion, this, or a convenient portion of it, was cut out and transferred into tubes of the "Soil Extract Solution." Fifty-six tubes were thus inoculated.

After two days, twenty-two tubes showed signs of growth which

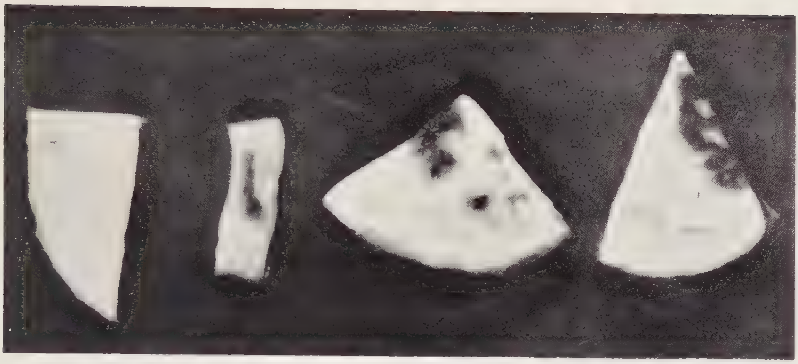

Fig. 1.

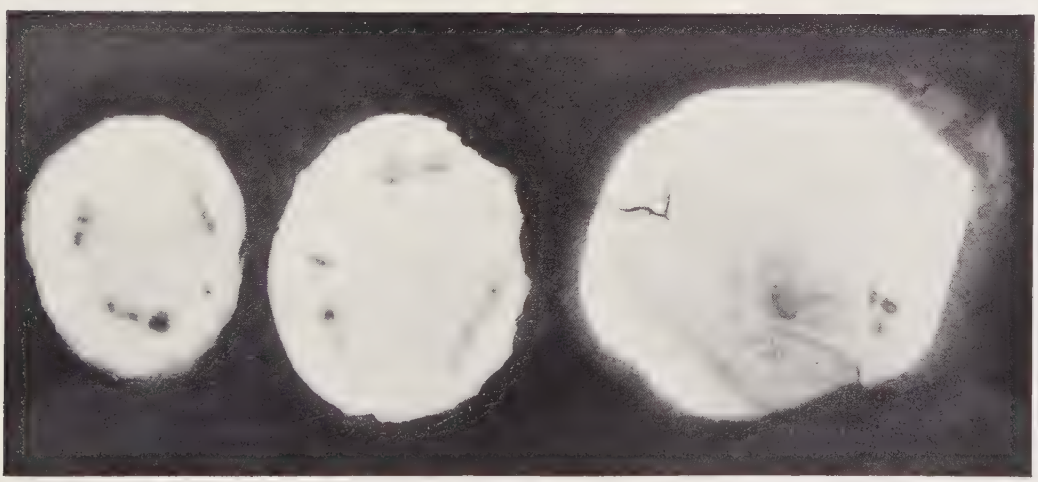

Fig. 2.

BURR.—Sprain or Internal Rust Spot of Potato (*B. rubefaciens*) (pp. 521–523).

proved to be a large rod-shaped bacillus. These tubes were therefore discarded. On the seventh day, six of the remaining tubes showed slight opacity in the manner of the original isolation cultures and were plated out in "Soil Extract Agar." No growth appeared on this medium even up to the twelfth day. The liquid cultures were, therefore, plated in nutrient gelatine. Growth on these plates appeared after five days as faintly yellow pin-point colonies consisting of Gram-negative, rod-shaped organisms measuring $1 \cdot 3\mu$ to $1 \cdot 8\mu$ long by $0 \cdot 5\mu$ broad. No colonies of any other type appeared on the plates. Pure cultures were obtained and subjected to the cultural tests detailed in the original paper on the subject. The organism proved to be identical with *B. rubefaciens*. No growth appeared in the remaining twenty-eight inoculated tubes.

The author regrets the omission in his original paper (1) of reference to Swellengrebel's earlier work (2) on the histology of "Sprain" lesions. In conclusion he wishes to thank Mr W. A. Millard, D.Sc., for his continued interest and encouragement throughout the work. His best thanks are also due to Mr J. Manby for the photographs.

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EXPLANATION OF PLATE XXXVIII.

Fig. 1. Extensive lesions mainly in the medulla of Golden Wonder tubers grown in soil inoculated with *B. rubefaciens*.

Fig. 2. Small lesions in cortex and medulla produced in tubers of Golden Wonder grown in soil inoculated with *B. rubefaciens*.

(*Received March 9th*, 1931.)

THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THE DEVELOPMENT OF THE ANGULAR LEAF-SPOT DISEASE OF COTTON

III. THE INFLUENCE OF AIR TEMPERATURE ON INFECTION

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(With Plate XXXIX and 3 Text-figures.)

In the first paper of this series (3) experiments of a preliminary nature were described dealing with the effect of air temperature and humidity on the development of the serious cotton disease variously known as "Angular Leaf-Spot," "Black-Arm" or "Bacteriosis." These experiments, though not extensive, and carried out under comparatively roughly controlled conditions, indicated the importance of these factors in influencing the development of the disease resulting from spray inoculation of young plants. In a later paper (5) an outline was given of the first experiments carried out in the more accurately controlled apparatus constructed under a grant from the Empire Marketing Board, details of which have been published separately (4). These first experiments dealt with the influence of soil temperature, and it was pointed out that while this factor had some effect on primary infection of seedlings, *i.e.* infection resulting from seed inoculation, this effect was not very great within the range of soil temperature at which cotton is usually grown. It was further shown that soil temperature had no detectable effect on secondary infection, *i.e.* infection arising from subsequent external inoculation, as by spraying the plants with a suspension of the organism.

The experiments have now been extended to include the effect of air temperature on such secondary infection of young plants and the results are described in the present paper.

The Rothamsted control chambers (4) consist essentially of heat-insulated and thermostatically controlled water-tanks, in which the soil tins for the plants are suspended and, fitting over these tanks, double-

walled glass air chambers, within which the temperature and humidity are automatically controlled. Illumination is provided by two 500-watt lamps in suitable reflectors suspended over each case, the heat from the lamps being absorbed by a screen of continuously flowing water.

The seed used throughout the experiments has been "Sakellarides" variety from the Gezira Plain, supplied by the courtesy of Mr R. E. Massey, Botanist to the Sudan Government.

Description of experiments.

Exp. 1. The forty-eight tins for the six chambers were filled with Gezira cotton soil and sown, in the glasshouse, with Sakel seed, two seeds in each tin. Before sowing the seed was treated with concentrated sulphuric acid for 15 minutes, to sterilise the outside. The plants were allowed to grow for 6 weeks, by which time they had produced two true leaves with two or three unfolding.

The six control chambers were started several days prior to the beginning of the experiment, the thermostats of the soil temperature tanks being set for a temperature of 22° C. (The temperature variation in the tanks is usually about 1° C.) The humidity controls were all set for 85 per cent. relative humidity. This experiment was carried out with an early type of humidity control (2) and the variation in humidity was somewhat great. Throughout the experiment, however, the humidity in all chambers was over 80 per cent. The thermostats of the air chambers were set for the following range of temperatures: 16, 20, 24, 28, 32 and 36° C. Unfortunately, a warm spell of weather began just at the time the experiment was started, and the lower temperatures could not be maintained, especially when the artificial lights were on. In these chambers, therefore, a variation of 4–5° C. resulted, and the average temperature of the six chambers during the experiment was 18–19, 22, 24, 28, 32 and 36° C.

The young plants were thoroughly sprayed by means of an atomiser with a strong suspension in sterile water of a virulent culture of *Bacterium malvacearum*, and placed in the chambers. Two tins in each chamber were left unsprayed as controls. Illumination was given for the rest of that day and night, and thereafter the plants received 16 hours' lighting daily from 5 p.m. to 9 a.m. The illumination was given during the night in order to allow the heating of the room due to the lights to be balanced by the drop in external temperature.

Infection was visible at the higher temperatures in 6–7 days and appeared complete in 12 days. The plants at the three high temperatures

were accordingly examined after this period and the amount of infection estimated. Infection was slower at the lower temperatures and these plants were left for a longer period, those at 24° C. for 19 days, and the remaining two chambers for 24 days, before final examination.

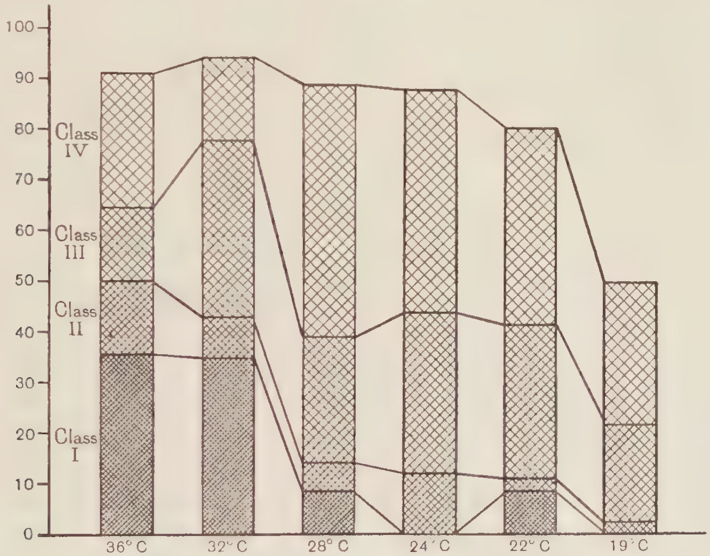
Table I.

Exp. 1. *Distribution of infection at various air temperatures.*

	36° C. Leaf no.				32° C. Leaf no.				28° C. Leaf no.				24° C. Leaf no.				22° C. Leaf no.				19° C. Leaf no.			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Plant no. 1	∞	∞	5	5	∞	10	15	1	—	10	7	5	—	—	35	11	—	0	52	10	0	0	15	0
„ 2	∞	∞	5	1	—	51	30	4	—	0	18	0	—	15	9	1	—	1	11	14	—	2	15	6
„ 3	∞	5	20	5	∞	∞	20	15	—	4	3	30	—	1	16	20	—	0	42	5	0	4	11	12
„ 4	∞	60	11	5	∞	—	10	15	—	12	7	5	—	—	16	1	—	—	65	14	0	0	14	0
„ 5	∞	∞	∞	20	—	—	5	5	—	—	4	4	—	0	5	0	—	7	15	5	0	1	0	0
„ 6	∞	25	25	4	∞	∞	∞	5	—	3	25	7	—	3	16	15	—	0	65	13	0	4	7	2
„ 7	∞	47	55	5	∞	75	30	2	—	∞	8	7	—	0	30	1	—	2	8	20	—	0	6	0
„ 8	∞	36	12	5	—	25	22	0	—	3	18	0	—	1	13	25	—	2	12	15	0	0	20	10
„ 9	∞	26	25	—	12	10	—	—	—	3	4	2	—	3	2	6	—	0	6	9	0	0	13	15
„ 10	15	6	5	0	∞	11	20	10	∞	18	13	5	—	—	—	—	0	3	24	8	0	1	7	10
„ 11	∞	∞	18	7	∞	∞	15	0	—	15	20	5	—	—	—	—	—	0	5	3	0	0	5	6
„ 12	23	28	0	0	—	—	—	—	—	∞	10	—	—	—	—	—	—	0	21	7	0	0	40	5
Total no. of leaves	48				37				36				25				36				46			
Class I 50 spots and over	17 (35·4 %)				13 (35·1 %)				3 (8·3 %)				0 (0·0 %)				3 (8·3 %)				0 (0·0 %)			
Class II 25 spots and over	7 (14·6 %)				3 (8·1 %)				2 (5·6 %)				3 (12·0 %)				1 (2·8 %)				1 (2·2 %)			
Class III 10 spots and over	7 (14·6 %)				13 (35·1 %)				9 (24·8 %)				8 (32·0 %)				11 (30·5 %)				9 (19·6 %)			
Class IV Less than 10 spots	13 (27·1 %)				6 (16·2 %)				18 (50·0 %)				11 (44·0 %)				14 (38·9 %)				13 (28·2 %)			
Total no. of leaves infected	44 (91·7 %)				35 (94·5 %)				32 (88·7 %)				22 (88·0 %)				29 (80·5 %)				23 (50·0 %)			

In the estimation of the disease an examination was made of each leaf, starting from the base of the plant, and counting the number of spots occurring on each separate leaf. A difficulty arises in such a method of estimation in that in many cases neighbouring spots tend to coalesce, forming a patch, in which it is difficult or impossible to distinguish the individual centres of infection (Plate XXXIX, fig. 2). This difficulty becomes even greater in the type of infection characterised by an attack of the leaf tissue on either side of a vein and extending along the vein (Plate XXXIX, fig. 3) (see also (1), Figs. 239 and 241). Clearly, in any attempt to estimate the *severity* of infection, as distinct from the *incidence* of infection obtained by mere counting of the number of diseased leaves, more weight must be given to such an extended lesion than to a single circumscribed spot. An arbitrary method of estimation was finally adopted of counting each patch, either of the "coalescence" type where individual centres of

infection could not be distinguished, or the "extended" type along a vein, as equivalent to five spots. This is obviously not exact, but provided the same method is used throughout, the estimation of severity of attack will be reasonably accurate. The results of this experiment are given in full in Table I. The control plants are not included in the table, as these were free from infection. Where more than about seventy-five spots or the equivalent in patches occurred on a single leaf the infection was recorded as indefinite (∞). In order to obtain an estimate of the total infection and its severity at each temperature the leaves were grouped



Text-fig. 1. *Exp.* 1. Percentage infection in four classes at various air temperatures.

in arbitrarily limited classes: Class I, severe infection, fifty spots or more; Class II, moderate infection, twenty-five spots or more; Class III, light infection, ten spots or more; Class IV, very light infection, less than ten spots. Text-fig. 1 shows the percentage infection in the four classes at the six temperatures. It is apparent that at the two higher temperatures, 32 and 36° C., there is little or no significant difference in infection, the slight reduction in the amount of heavy infection (Classes I and II) at 32° C. being balanced by the increased amount of light infection (Class III). Below 30° C., however, there is a decided fall in the amount and severity of infection with decreasing temperature. From the figures in Table I it is clear that the apparent increase in severe

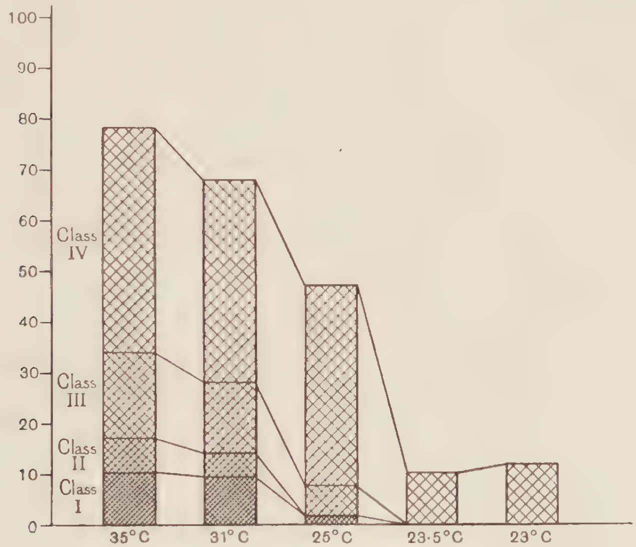
infection at 22° C. is hardly significant, the three leaves having over fifty spots only just falling within Class I.

It should be noted here that the plants used were small and at the time of spraying only two true leaves were fully unfolded and even these were still developing. This is of importance in view of the distribution of the disease on the plant. It will be noted from Table I that at the two higher temperatures infection was most severe on the basal leaves, decreasing in intensity on passing up the plant. In later experiments somewhat older plants were used, and in most cases the two or three basal leaves were more or less fully developed. It has been shown by other workers and confirmed by the writer that *B. malvacearum* normally attacks only developing tissues, and this would explain the lack of infection of the basal leaves in later experiments. In the present experiment also it is highly probable that the lack of infection of the basal leaves at the lower temperatures may be due to the slowness of development of the disease at these low temperatures and the consequent maturing of these leaves before the disease has progressed far enough to produce a lesion. Further development is then arrested. Reference to this variation in distribution of the disease will be made in the discussion (p. 532).

Exp. 2. This experiment was in essentials a repetition of the first with certain modifications. The plants were raised as before in the glasshouse in Gezira soil with four plants per tin. When three to four true leaves were unfolded the plants were sprayed with a water-suspension of the bacteria and placed in the chambers. The soil-temperature thermostats in all chambers were set for 28° C. and the humidity controls for 85 per cent. relative humidity. The air thermostats were set for the following range of temperatures 40, 35, 30, 25 and 20° C., while one chamber was left unheated to run at air temperature. As in the previous experiment it proved impossible to maintain the lower temperature, while the highest temperature showed a fluctuation from 38 to 40° C. The actual mean temperatures derived from the thermograph charts were therefore, 39, 35, 31, 25, 23·5 and 23° C. Illumination was provided for 16 hours out of the 24.

After 16 days it was found that only a negligible amount of infection had occurred, and it seemed certain that the culture of *B. malvacearum* used had lost a considerable degree of virulence. As the plants had made good new growth during the period in the chambers and most of the basal leaves had dropped off, the plants were re-sprayed with a strong suspension of a newly isolated culture of the same strain which had been maintained on experimental plants in the glasshouse and had thus

retained its full virulence. Good infection resulted, and appeared complete after 14 days at 35, 30 and 25° C. The plants at 39° C. had made little or no growth in the chambers and after this further period of 14 days were dead. The plants at the two lower temperatures were left for a further 7 days to allow of full development of any infection.



Text-fig. 2. *Exp.* 2. Percentage infection in four classes at various air temperatures.

Table II.

Exp. 2. *Distribution of infection in four classes at various air temperatures.*

	35° C.		31° C.		25° C.		23·5° C.		23° C.	
	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%
Total no. of leaves	88	—	75	—	68	—	60	—	51	—
Class I, 50 spots and over	9	10·2	7	9·3	1	1·5	0	0·0	0	0·0
,, II, 25 ,, ,,	6	6·8	4	5·3	0	0·0	0	0·0	0	0·0
,, III, 10 ,, ,,	15	17·0	10	13·3	4	5·9	0	0·0	0	0·0
,, IV, less than 10 spots	39	44·3	30	40·0	27	39·7	6	10·0	6	11·7
Total no. of leaves infected	69	78·3	51	67·9	32	47·1	6	10·0	6	11·7

Estimation of infection was carried out on the same basis as in the previous experiment. The distribution of the infection into the four arbitrary classes at the various air temperatures employed is summarised in Table II and shown in graphic form in Text-fig. 2. It will

be seen that, in general, the results of this experiment are the same as in the previous test, infection occurring most freely at temperatures above 30° C. and falling off rapidly in intensity below this point.

Exp. 3. In further confirmation of these experiments it may be well to give here the relevant figures of an experiment which will be included at greater length in a later paper. This experiment is one of a series to be carried out dealing with the effect of alternating conditions on the disease. One of the chambers was arranged to give an alternating air temperature, while the remaining five were run at constant temperatures covering the extreme range of this chamber. As the conditions in these five cases were identical with the experiments described above they are relevant to this discussion.

As before the tins were filled with Gezira soil and sown in the glasshouse with four seeds per tin. When of a suitable size the plants were transferred to the chambers for a few days and then sprayed with the organism. The mean temperatures of the five chambers were 39, 35, 30, 25 and 23·5° C. As in Exp. 2 the plants at 39° C. made no growth and many were dying at the close of the experiment. No definite infection was detectable on the leaves of these plants, and they are omitted in the further discussion.

One further modification in this experiment aimed at testing the influence of time of spraying the plants in relation to the time of illumination. The plants in one half of each chamber were sprayed 30–60 minutes after the lights had been switched on, and the remaining plants on the following morning 30–60 minutes after the lamps were extinguished. As in the previous experiments infection developed rapidly at the higher temperatures and more slowly at the lower. The plants at 35° C. were examined after 21 days and the remainder 3 days later. The results are again summarised in Table III, and shown graphically in Text-fig. 3.

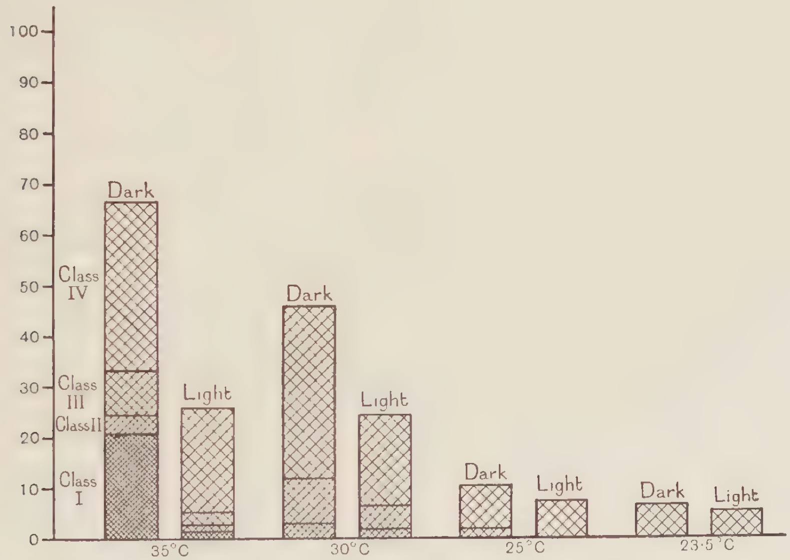
Table III.

Exp. 3. *Distribution of infection in four classes at various air temperatures.*

	35° C.				30° C.			
	Dark		Light		Dark		Light	
	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%
Total no. of leaves	78	—	78	—	68	—	62	—
Class I, 50 spots and over	16	20·5	1	11·3	0	0	0	0
„ II, 25 „ „	3	3·9	1	1·3	2	2·9	1	1·6
„ III, 10 „ „	7	8·7	2	2·6	6	8·8	3	4·8
„ IV, less than 10 spots	26	33·4	16	20·5	23	33·9	11	17·8
Total no. of leaves infected	52	66·5	20	25·7	31	45·6	15	24·2

Table III (*cont.*).

	25° C.				23·5° C.			
	Dark		Light		Dark		Light	
	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%
Total no. of leaves	59	—	55	—	64	—	60	—
Class I, 50 spots and over	0	0	0	0	0	0	0	0
,, II, 25 ,, ,,	0	0	0	0	0	0	0	0
,, III, 10 ,, ,,	1	1·7	0	0	0	0	0	0
,, IV, less than 10 spots	5	8·5	4	7·3	4	6·3	3	5·0
Total no. of leaves infected	6	10·2	4	7·3	4	6·3	3	5·0



Text-fig. 3. *Exp.* 3. Percentage infection in four classes at various air temperatures.

Considering first the effect of temperature it is clear that the results of this experiment agree very closely with those of Exp. 2. There is the same high degree of infection at 35° C. with rapid decrease in the amount of disease at progressively lower temperatures. This is true of both series, considered separately, whether sprayed in the light or in the dark. The comparison between the two series, however, is striking. At all four temperatures a higher degree of infection resulted from spray inoculation in the dark than on the corresponding plants which were sprayed in the light. At the two higher temperatures this influence of time of inoculation is very marked. This point will be discussed later.

Discussion of results.

Comparison of the results of the air-temperature experiments outlined here with those on the influence of soil temperature previously described (5) shows that the effect of temperature in the two cases is opposite. In the case of primary infection of the seedling resulting from seed inoculation, high soil temperatures at the time of germination materially reduce the amount of disease, while in the case of secondary infection produced by spray inoculation high air temperatures favour the development of the disease, and low temperatures almost completely inhibit it. The optimum temperature for growth of the parasite in pure culture is about 27° C., while growth stops almost completely above 35° C. With the exception of one soil-temperature experiment this optimum does not agree with the temperature for maximum infection in either series of experiments. It seems possible that in the case of high soil temperature there is a direct effect on the parasite on the seed coat, preventing its multiplication and reducing its vitality before germination of the seed. This is supported by the fact that in the soil-temperature experiments, where the organism was introduced inside the seed coat, and was thus in contact with the embryo from the beginning, 100 per cent. infection resulted, even at a soil temperature of 40° C. In the case of the air-temperature experiments, where the parasite comes immediately into direct contact with the leaves, entrance through the stomata probably occurs within a very short time. It appears that the temperature relations of *B. malvacearum* within its host are different from those in pure culture or on some non-living surface such as the exterior of the seed, and that once penetration of the tissues has occurred a high temperature favours the development of the disease. The effect of temperature then becomes a problem of the balance between the physiological resistance of the tissues of the host, conditioned by the rate of growth and maturation and the biochemical reactions as influenced by the environment, and the activity of the parasite, itself in turn affected by the temperature. A clue to the nature of the biochemical factors that influence the development of the disease is given by a rough test carried out on the amount of reducing sugars present in the leaves at various temperatures. The work was done by Dr F. G. Gregory, to whom the author's thanks are due. The means of these estimations, which were done only on a small scale were: 23·5° C., 1·4 per cent.; 25° C., 2·6 per cent.; 31° C., 6·5 per cent.; 35° C., 6·9 per cent. It will be seen that there is a close correlation between these figures and the

figures for infection at the corresponding temperatures. Further work on this point is planned.

The other interesting point in these experiments is the effect of time of inoculation. Contrary to expectation the disease developed much more severely on leaves sprayed in the dark than on those in the light. Since entrance takes place through the stomata it would have seemed probable that infection would occur more readily in the light when the stomata are open. The explanation may be in the water relations of the host. During the light period it is reasonable to suppose that the rapid transpiration will result in a less amount of water either as liquid or vapour in the intercellular spaces and especially in the sub-stomatal chamber, whereas in the dark the amount of water present will be increased. The bacteria would then have greater opportunities for active motility and would enter the stomata more readily and in greater numbers during this period.

Summary.

Experiments carried out in the Rothamsted control chambers on the influence of air temperature on the angular leaf-spot disease of cotton plants, resulting from spray inoculation of young plants, show that high air temperatures favour the development of the disease. Maximum infection occurs at an air temperature of 35–36° C. with decreasing incidence at progressively lower temperatures. At a constant air temperature of 39–40° C. cotton plants make no growth and eventually die.

Infection takes place more readily when the inoculation is carried out during the non-illuminated period.

The relation of these results to the experiments on the influence of soil temperature is discussed.

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EXPLANATION OF PLATE XXXIX.

Cotton leaves affected with angular leaf-spot disease, showing types of infection. Photographed by transmitted light.

Fig. 1. Type of infection characterised by discrete spots.

Fig. 2. Type of infection characterised by coalescence of spots.

Fig. 3. Type of infection characterised by extension along vein.

Fig. 4. Leaf showing "coalescence" and "extended" types of infection.

Fig. 5. Leaf showing severe infection described as "indefinite."

(*Received February* 15*th*, 1931.)

STOUGHTON.—THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THE DEVELOPMENT OF THE ANGULAR LEAF-SPOT DISEASE OF COTTON (pp. 524–534).

LEAF-SPOT OF OATS, *HELMINTHOSPORIUM AVENAE* (BRI. AND CAV.) EID.

By D. M. TURNER, B.Sc.

(*Demonstrator in Agricultural Botany, University of Leeds*),

AND W. A. MILLARD, D.Sc.

(*Lecturer in Agricultural Botany and Adviser in Mycology, University of Leeds*).

(With Plates XL and XLI and 4 Text-figures.)

CONTENTS.

Introduction and review of the literature.

This investigation was undertaken for two reasons. First, complaints from farmers in Yorkshire of a poor "take" of oats occur with almost depressing regularity after every cold spring, and from examination of the seedlings this failure has appeared to be due to leaf-spot. Secondly, reference to the literature left us in considerable doubt as to the life history of *Helminthosporium avenae*, as well as of the symptoms and extent of its attack, and in complete ignorance of the origin of the disease or of means for its control.

The *Helminthosporium* diseases of cereals have been studied by many investigators, but most of the work has been done on wheat and barley rather than on oats. From Drechsler's (2) summary we find that Briosi and Cavara reported an oat-spot in Italy in 1889, characterised by narrow, elongated, olive-green lesions with dark margins, commencing

at the tip of the leaf. *Helminthosporium* was isolated and designated as *H. teres* (Sacc.)—*Avenae Sativae*. In 1891, Eidam identified a *Helminthosporium* spot on oats which regularly attacked the first, second and third leaves, and which was not pathogenic to wheat or barley. This he claimed as an independent species of *H. avenae*. Ritzema Bos in Holland (1900), Ravn (10) in Denmark (1901) and Johnson (4) in America (1914) have all described a *Helminthosporium* attack upon oats, but with great variation in the type of lesion produced. Bos attributed a short round foliar spot associated with a red colour in the attacked leaves to *H. gramineum*, and Johnson (4) a stripe form of lesion, which he also ascribed to *H. gramineum*.

In 1901 Ravn described the symptoms of the attack of *H. teres* and *H. gramineum* on barley and of *H. avenae* on oats, and differentiated between these three species. He directed his attention mainly to the barley infections and his description of the attack of *H. avenae* is in terms of that of *H. teres* on barley. The latter, he says, "appears first in fully developed and unfolded leaves as short lesions, which are never in stripes, the leaves are never torn or limp, the leafy sheaths are slightly attacked in isolated spots, and the number of lesions per leaf varies from 1 to 100." "*H. avenae*," he states, "agrees with this description, with the additional points that the spots are of a vague grey to greyish brown, that no distinct network is to be found in the lesions and that a reddish colour may develop at the fading stage. The attack is most severe in young seedlings and on the autumn stubbles." From this it is seen that Ravn definitely stresses the absence of the stripe form commented upon by other workers and frequently obtained by the present writers; moreover, the figures given for the maximum number of lesions per leaf are far in excess of any found throughout the course of this work.

Drechsler (2), in 1923, published a detailed study of the *Helminthosporium* genus. He describes *H. avenae* on oats as broad and irregular or long and narrow spots with no reticulation, there being rarely more than three or four per leaf. The margins may be definite or ill-defined when they merge gradually into yellow or red, and spread over large areas of the leaf. Extensive spotting may be absent, in which case there is premature withering similar to that caused by weathering or maturation. On the death of the leaf the red colour fades, leaving pale yellow or grey areas bearing conidia. This author agrees with Ravn that there are two definite stages in the disease—the primary or seedling stage and the secondary or adult stage, arising from infection by the primary

spores. He finds, with Briosi and Cavara, that there is a marked similarity between the spores of *H. avenae* and *H. teres*, and finally suggests that these might be considered as biological forms of the same species. It would scarcely seem justifiable to interpret the "long and narrow spots" included in Drechsler's definition of the disease as stripes. Yet, in 1923 Smith (13) and again in 1925 the same author (recorded by Parker (8)) reported the presence of a stripe form of *H. avenae*, which may quite well have been identical with the stripe which Johnson (4) attributed to *H. gramineum*.

As will be shown later, the present writers' experience is confirmatory of Smith's in that they find a definite stripe is commonly produced in this disease. This stripe is indeed so clearly a stripe and not a narrow spot that, in their opinion, the name leaf-stripe would be a far better cognomen of the disease than leaf-spot. From this survey, however, it is obvious that there is little agreement among previous investigators as to size, form or number of the lesions produced by this disease.

Our present knowledge of the mode of attack is equally unsatisfactory. Ravn, in his work upon barley, suggests that there is a strong analogy between the *Helminthosporium* mode of attack and that of the smuts, but this theory has recently been disposed of, as far as *H. gramineum* is concerned, by Smith (14). There are no direct records of the origin or progress of the attack by *H. avenae*.

Table I.

Variety	District grown	% spore infection (*H. avenae*)
Beseler's Prolific	Not known	9
Victory	,,	17
Victory	Forfar	16
Yielder	Not known	62
Longhoughton	Berwick	23
Black Tartarian	Forfar	16
Abundance	E. Lothian	36
King	,,	15
Longhoughton	Aberdeen	49
Victory	,,	28
Beseler's Prolific	Not known	19
Victory	,,	11
Victory	,,	6

Damage to the oat crop by *H. avenae* appears to be widely distributed. In U.S.A. (6) it is reported as causing extensive root and foot-rot damage. Ravn found it of economic importance in Denmark and it has been periodically recorded in Germany (3). More recently it has

been held to be responsible for considerable loss in the "braird" of seedling oats in Westphalia (11). In the British Isles it appears regularly, with varying severity, over widespread areas but especially in the Northern counties of England, and in the West of Scotland, where the attacks are said to be so severe that they frequently cause a total failure of the crop. A systematic examination of samples of Scotch grown oats supplied us by two well-known and reputable firms of seedsmen has been made, the results of which are given in Table I. It will be seen that the percentage of infection is high and that seed from these districts provides a very effective means for the distribution of the disease.

Symptoms of the disease.

Leaf-spot appears primarily as a seedling disease, the first indication being seen about the tenth day after sowing as dark brown streaks or spots on the coleoptile (Plate XL, fig. 1). These cannot, however, be taken as conclusive evidence of infection, as there is a tendency in many varieties of oats for the vascular bundle of the coleoptile to darken at this stage in the normal course of development. The most marked appearance of the disease occurs in the first foliage leaf which may show certain characteristic modifications, *e.g.* it may emerge at right angles to the coleoptile, or at any angle between this and the vertical; dwarfing may occur so that the blade becomes wider in proportion to its length; the edges of the leaf may undulate and become so twisted that some considerable distortion is produced (Plate XL, fig. 1). Lesions numbering from one to six arise above the junction of the blade and sheath and develop as light chlorotic areas of irregular shape and size with reddish brown centres. They lie towards the edge of the lamina, thus forming the primary cause of the undulations. As the leaf extends the lesions increase in size and number and may also appear on the sheath. From six to ten spots per leaf is common, but in a severe attack the number may extend to twenty. Even at this early stage the development of a stripe emphasised by Smith is by no means rare, but it always arises primarily as a row of isolated spots which later anastomose (Plate XL, fig. 2).

At the end of 21 days the lesions produce purplish brown to grey centres which may be punctured; they may have either a well-marked red-brown margin or spots of colour scattered over the surface, and are generally surrounded by a lighter halo which merges gradually into the normal green of the leaf. There is no reticulation as in barley net blotch

(*H. teres*), and the chlorosis surrounding the lesions tends to lie between the main veins and to be restricted by them. "Striping" is of common occurrence at this stage, but is never accompanied by the splitting and shredding characteristic of barley stripe (*H. gramineum*). Similar lesions may appear on the second leaf as it emerges and also on the third, but in no case, in all the plants examined, has the seedling infection been found to extend beyond this.

At the end of 28 days, if the infection is heavy, the diseased leaves become limp, pendant and shrivelled (Plate XL, fig. 2); they turn greyish yellow and subsequently the seedling dies. Conidia are now produced abundantly upon the dying leaves. If the attack is mild and only the first leaf is affected the seedling may grow without any apparent check, but where the second and third leaves are badly attacked death usually follows.

Both Ravn and Drechsler considered that the disease shows two well-marked phases: a primary or seedling infection which is seed borne and a secondary or adult infection produced by spores developed from the primary stage. The writers' observations confirm these opinions, secondary infection occurring somewhat sparsely during 1929 and very abundantly in 1930. These secondary lesions resemble those found on the seedlings except that they are larger and of a deeper red colour (Plate XLI, fig. 5). They are comparatively few in number, varying from one to five per leaf. They may be present on all the leaves and sheaths above the fifth and are very commonly found on the uppermost. The affected leaves wither more quickly than healthy leaves, but during a normal season the effect on the crop is apparently negligible. This stage, however, is the source of the spores which infect the developing grains and carry the infection into the succeeding crop. It has been found that under natural conditions spores are produced most rapidly during periods of high humidity, and the amount of secondary infection is, therefore, to a great extent dependent upon the season. Thus, heavy primary infection does not necessarily involve an extensive secondary attack; a period of warm, dry weather after the plants reach the third leaf may indeed reduce the secondary stage to a minimum. Conversely, a cool, wet period will cause both stages of the disease to spread rapidly.

Seed infection.

Seed infection may be that of mycelium only, which is common, or of spores of the fungus which is far less common. An examination

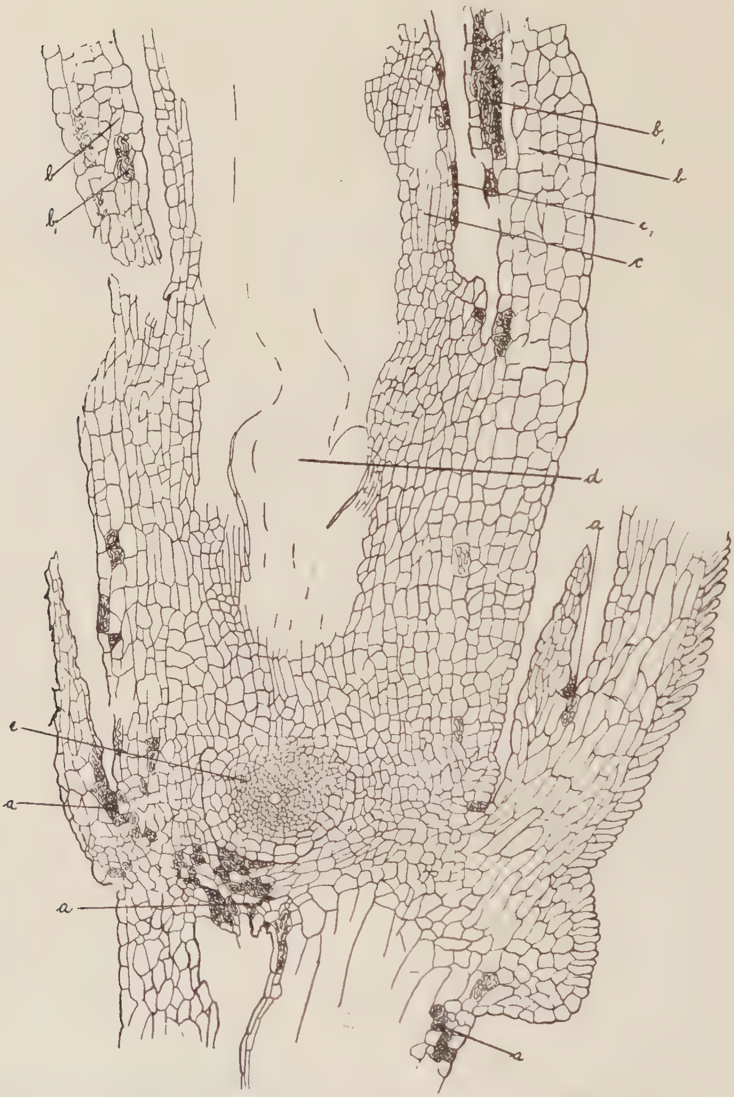
has been made during the last two years of samples taken from six infected crops. In these, from 3 to 37 per cent. of the grains showed spore infection, but in no case were the spores abundant on any one grain. Mycelial infection, however, was found in 80 to 100 per cent. of the grains in the different samples. The mycelium lies on the inner side of the glumes and within the cells of the pericarp (Text-fig. 1). It is dark, thick-walled, highly septate, irregular and intracellular. It tends to lie against the long axis of the cells, but travels transversely in an oblique direction and resembles very closely the submerged forms of

Text-fig. 1. Pericarp of infected grain. (*a*) base of hair, (*b*) intracellular mycelium. Drawn under the camera lucida (× 600).

mycelium developed in culture. In the pericarp there is a tendency towards aggregation round the base of the hairs which may suggest these regions as possible points for penetration. No ascigerous stage of the fungus has been found on the infected grains or in culture; the chief sources for the spread of the disease, therefore, are the spores and the internal mycelium. The viability of the mycelium within the pericarp is well marked and extends over a long period; infected seeds from the 1927 crop still produced diseased plants three years later. It is probable, therefore, that this mycelium is of much greater importance in carrying the disease to the next year's crop than are the spores adhering to the outside of the grains.

HISTOLOGY OF THE INFECTED SEEDLING.

The development of the primary infection is much more clearly associated with the mycelium within the pericarp cells and palea than with the adhering external spores. Detailed microscopic examination of microtome sections taken through the base of the embryo 11 days after sowing shows that infection takes place before the coleoptile emerges from between the palea and that it arises from within and not from without the palea. Any penetration of the coleoptile by mycelium or germinating spores after its emergence is extremely unlikely to produce disease symptoms. There appears to be a very critical infection period during the germination of the seed, which under ideal conditions of germination is very short. The extent of the primary attack is dependent on the lengthening of this period by adverse conditions. On germination, the dormant mycelium spreads towards the embryo and penetrates it at those points where the cells offer the least barriers, *i.e.* at the junctions of the scutellum and the epiblast (Text-fig. 2, *a*). There is also a certain amount of entry effected through the external side of the lower part of the scutellum where the epithelial cells are less closely packed. From these points the fungus spreads upwards to the cells of the coleoptile, inwards towards the rudimentary coronal roots and downwards to the seminal roots where it may produce the "foot rot" of the American workers. The critical region for the infection lies, however, in the cells of the coleoptile (Text-fig. 2, *b*) where the passage of the hyphae through their wide lumen is comparatively easy. The fungal threads move towards the rolled primary leaves and penetrate the first leaf on its outer edge (Text-fig. 2, *c*). Meanwhile the leaf is elongating and the cells are being withdrawn from the region of infection so that the lesions develop as small scattered areas, lying in rows which may later join to produce the stripe form. Where there is extensive infection in the coleoptile cells and where growth is delayed, the hyphae will pass through the first leaf into the second and third and may reach the growing point, thus causing the death of the plant. If growth conditions improve, however, the fourth and subsequent leaves are lifted out of the infected zone before the third leaf is penetrated, and the primary stage does not extend beyond this leaf. In this case, there may be infection and destruction of some of the tillers with consequent loss of vigour to the plant. Inoculation experiments show that external penetration through the cuticle of the coleoptile is both difficult and slow, and that the growing point and developing leaves outgrow the region of infection



Text-fig. 2. (a) Points of penetration of the fungus through scutellum and epiblast. (b) Cells of the coleoptile. (b_1) Mycelium within coleoptile cells. (c) Cells of the first leaf. (c_1) Mycelium within the cells of the first leaf. (d) Region of the growing point. (e) Rudimentary cereal root. Drawn under the camera lucida (× 400).

before the hyphae can pass through to the inner surface. The fungus does not move rapidly through the embryonic tissues in the primary stage and is located in the coleoptile chiefly in scattered groups of cells rather than in any one extensive region. It is on account of this slow rate of progress that the plant is able under favourable conditions to outgrow the disease. Abundant spores are produced on the lesions after the death of the leaf, usually at the time when the plant is in the fifth or sixth leaf, and the secondary stage is then initiated by the penetration of these germinating spores. The lesions thus produced are scattered indefinitely over the leaf as somewhat irregular areas with a tendency towards striping, due to the limiting effect of the larger veins. The invading hyphae resemble the short, broad, much septated forms previously described in the pericarp and coleoptile cells, and move slowly from cell to cell chiefly in a horizontal direction. There is no indication of the retention of mycelium within the sheaths enveloping the younger leaves as described by Smith for *H. gramineum*. There is, in fact, a clear-cut gap between the two phases of infection, during which time the spores which are solely responsible for the secondary stage are developed.

The spores produced from these lesions, especially those on the upper leaves, are carried on to the open palea during the flowering of the spikelet. Here they germinate and penetrate the pericarp of the maturing grain, to remain in a dormant condition until the seed germinates and thus complete the cycle of infection.

Isolation of the fungus and cultural characteristics.

The fungus was easily isolated in single-spore culture from the spores developed on the primary lesions after incubation at 24° C. under very moist conditions for several days. It is an interesting fact that the conidiophores are frequently produced on the leaves where no apparent lesions exist.

Considerable diversity of opinion exists as to the ease with which the various species of *Helminthosporium* produce spores in artificial culture, and since this character may be of importance in distinguishing between these species the present writers have given it attention. Ravn (10), working upon *H. gramineum*, *H. teres* and *H. avenae*, found conidia formation rare upon his media. Drechsler (2) claims that *H. teres* spores easily on Beijerinck's agar and on tap-water agar, but omits any reference in this connection to *H. avenae*. Stevens (15) was unable to

obtain spores of *H. avenae* on any artificial media excepting sterilised wheat straw, where sporing occurred on one occasion. Paxton (9), working on *H. gramineum*, reports considerable sporulation on corn-meal agar, a statement which Johnson (5) was unable to confirm. Claims have been made by contemporary workers on *Helminthosporium* that *H. avenae* spores freely on potato agar, but the present writers have never obtained spores on this medium. *H. sativum*, however, does not appear to present any difficulties in sporulation. It spores freely on most media, but Stevens finds that an increase in carbohydrate content has a marked effect upon the rate of spore development. In view of these conflicting statements it was obviously desirable to make a detailed study of our *H. avenae* culture. A wide range of media has been used to embrace varying carbohydrate and nutrient contents, hydrogen-ion concentration and sterilisation methods. They may be divided into the following groups:

(*a*) High carbohydrate content.
(*b*) Low nutrient content.
(*c*) Synthetic media.
(*d*) Plant tissues.

Variations in *p*H and in the method of sterilisation are included in each group.

(*a*) *High carbohydrate content.*

1.	Prune-juice agar	*p*H 6·1	Intermittent steaming at 100° C.
2.	Beer-wort agar	*p*H 6·4	,, ,, ,,
3.	Nutrient-sugar[1] agar	*p*H 6·1	,, ,, ,,
4.	Quaker-oat agar (unfiltered)	*p*H 6·3	Autoclaved for 20 mins. at 115° C.
5.	Potato agars:		
	Potatoes unpeeled and filtered	*p*H 6·1	Autoclaved for 20 mins. at 115° C.
	Potatoes unpeeled and unfiltered	*p*H 6·2	Intermittent steaming at 100° C.
	Potatoes peeled and filtered	*p*H 7·0	Autoclaved for 20 mins. at 115° C.
	Potatoes peeled and unfiltered	*p*H 6·2	Intermittent steaming at 100° C.
6.	Nutrient-potato agar	*p*H 7·0	Autoclaved for 20 mins. at 115° C.

(*b*) *Low nutrient content.*

1.	Corn-meal agar[2]	*p*H 6·0	Autoclaved for 20 mins. at 115° C.
2.	,, ,,	*p*H 7·0	,, ,, ,,
3.	,, ,,	*p*H 7·7	,, ,, ,,
4.	Washed tap-water agar	*p*H 6·9	,, ,, ,,

[1] Laevulose 2·0 per cent., dextrose 1·5 per cent., sucrose 1·0 per cent. Based on the analysis of the sugars present in oat leaves.

[2] *U.S.A. Dept. Agric. Bur. Pl. Indus. Bull.* 131 (1913), pp. 3–18.

(*c*) *Synthetic media.*

1. Beijerinck's[1] agar	*p*H 6·5	Intermittent steaming at 100° C.
2. Tyrosinate agar	*p*H 6·9	,, ,, ,,
3. Dextrose agar	*p*H 6·8	,, ,, ,,
4. Calcium-malate agar	*p*H 7·0	,, ,, ,,

(*d*) *Plant tissues.*

1. Potato plugs	Autoclaved for 20 minutes at 115° C.
2. Mangel plugs	,, ,, ,,
3. Young oat seedlings	,, ,, ,,
4. Mature oat leaves	,, ,, ,,
5. Mature oat stalks	,, ,, ,,

6. Oat glume, palea, and seed agar, *p*H 6·4, autoclaved for 20 minutes at 115° C.
7. Young oat leaves, sterilised in 0·1 % $HgCl_2$ for 30 minutes and well washed in sterile water.
8. Young oat shoots as (7) laid on tap-water agar as suggested by Stevens(15).

Certain differences in the type of growth were found in the above groups, the chief of these being in the amount of dark submerged mycelium formed. In group (*a*) there was a marked increase in the aerial mycelium which is white and fluffy with arborescent tufts, and in the dark green submerged growth which appeared after 7 days in all the media except beer-wort and potato agars. In group (*b*) there was a reduction of aerial growth which became hyaline, less woolly and showed less of the characteristic tufting. The submerged form was absent on tap-water agar and was reduced on the corn-meal, especially at *p*H 7·7. In both of these groups there were indications of zonation which was very strongly marked on all the media except beer-wort, potato and tap-water agars.

In group (*c*) there was great variation in the amount of growth, but the presence of sugars and organic salts appeared to increase the amount of submerged mycelium. Zonation was absent.

In group (*d*) some interesting features arose. On oat tissues sterilised by heat the aerial mycelium was sparse and slow in development, but where the death of the leaves was not involved, that is, where external fungicides had been used, the aerial mycelium developed abundantly in characteristic arborescent tufts. This was followed by a darkening of the hyphae and the production of modifications strongly resembling the submerged forms. On mangel and potato plugs abundant growth of both types of mycelium occurred, and on a combination of tap-water agar and sterile oat shoots a similar growth was obtained.

[1] NH_4NO_3 0·5 gm., K_2HPO_4 0·2 gm., $MgSO_4$ 0·2 gm., $CaCl_2$ 0·1 gm., ferrous sulphate trace, distilled water 1000 c.c., agar 15 gm.

With the exceptions quoted, neither differences in the pH of the medium nor variations of sterilisation methods produced marked changes in growth.

No sporulation occurred on any of the cultures excepting on sterilised oat leaves. Here, in one tube of autoclaved oat leaves, a single conidiophore bearing five conidia appeared after 21 days' incubation, whilst on young oat leaves sterilised with mercuric chloride several conidia developed. The latter appeared only when the plate cultures were kept very moist with sterile water, and then only sparsely. None of the spores so produced proved viable.

A method for spore production.

It is evident from the foregoing results that conidia cannot be freely developed in culture, nor upon sterilised living tissues when severed from the plant. We have found that the only means whereby spores may be obtained freely is to carry the disease through young seedlings grown under aseptic conditions and to obtain the spores from the lesions thus formed. This was effected as follows:

5 c.c. of tap water are placed in a test-tube and two pieces of glass tubing, sufficiently long to project above the level of the water, are inserted. A loose pad of surgical gauze is placed on the glass tubing so that the pad, whilst remaining moist, does not become saturated. The tubes are lightly plugged and sterilised. The pad is then heavily inoculated with mycelium of the fungus, together with some of the medium in which it has grown, and the tubes incubated for 5 to 6 days at 24° C. At the end of this time the upper portion of the gauze will be thoroughly impregnated with the fungus. Four dehusked oat seeds, sterilised by Jensen's hot-water method, and in addition, by immersion into $\frac{1}{8}$ per cent. formalin for 15 minutes, are placed upon each pad and the tubes are removed to a greenhouse with a maximum day temperature of 55° F. At this temperature the growth of the seedlings is sufficiently retarded for the fungus to penetrate and infect them in the early stages. The typical lesions appear on the coleoptile and first leaf, and the shoots are then removed aseptically to a sterile Petri dish and incubated at 24° C. under moister conditions than exist in the tubes. By this means a sufficient supply of spores for inoculation experiments can be obtained. Fourteen tubes were prepared in this way, twelve of which were inoculated and the remaining two acting as controls. The results are embodied in the following table:

Table II.

	No. of tube												Control	
	1	2	3	4	5	6	7	8	9	10	11	12	(a)	(b)
No. of seeds	4	4	4	4	4	4	4	4	4	4	4	4	4	4
No. germinated	3	4	4	2	4	3	4	4	4	3	2	4	4	4
Infected plants	3	4	3	2	3	3	4	4	3	3	1	4	0	0

It will be seen that in the twelve inoculated tubes forty-one out of the forty-eight or 85 per cent. of the seeds germinated, and that of these thirty-seven of the seedlings or 90 per cent. were infected. The controls showed no sign of disease. This method has been found to be distinctly superior to the "Rag Doll" method advocated by Stevens. It has the advantage that by it the air supply, temperature and moisture are more easily controlled and the growth of the seedlings can be sufficiently retarded to allow of strong infection in most cases.

Pathogenicity of the fungus.

"Marvellous" white winter oats have been used for all the inoculation experiments, the grain being previously sterilised by Jensen's hot-water method, and by subsequent immersion into $\frac{1}{8}$ per cent. formalin for 1 hour. As this work was carried out during the winter months all the plants were grown in a cool greenhouse, and some difficulty was experienced in the drying out of the mycelium when used as inoculum. This was obviated by growing the plants in groups of four under open glass cylinders 2 in. × 18 in., with the upper end lightly plugged with cotton wool. Support was given by canes driven into the soil before the seeds were sown. Three such cylinders were arranged in a 12 in. pot, so that twelve plants could be inoculated.

The inoculations were divided into two groups: in the one mycelium only was used as inoculum, and in the other spores produced under aseptic conditions were used. In group I inoculations were carried out both upon the coleoptiles and the first foliage leaves, and in group II upon the foliage leaves only.

Group I.

(*a*) *Coleoptile inoculations with mycelium.*

The coleoptiles of twelve plants were inoculated when from 1·0 to 1·5 cm. high from a sixteen-day culture; in six plants the epidermis was

slightly ruptured before inoculation and in the other six it was not ruptured. Within 2 days the typical white-tufted mycelium was developed at the point of inoculation, and after 8 days ten plants showed faint brown lines in the tissues above and below this point, these lines being more definite where the epidermis had been ruptured. After 18 days, although the lines had extended and darkened in colour, no indication of lesions could be found on the developing leaves. The coleoptiles of six plants were removed and incubated under moist conditions, and from five the typical spores of *H. avenae* were produced. Seventy days later the leaves still showed no trace of the disease and the coleoptiles died down in the normal way. The controls throughout remained healthy. Whilst, therefore, infection had undoubtedly occurred, the penetration of the fungus through the cuticularised surface of the coleoptile epidermis is so slight as to be almost negligible.

(*b*) *First foliage leaf inoculations with mycelium.*

Twelve plants were used in this series, in eight the leaves remained intact, and in four the epidermis was ruptured by lightly rubbing the surface with a cheese cloth. By this method excessive damage to the leaf is avoided. Lesions appeared on eleven of the twelve inoculated plants within 8 days, but there was no obvious difference between those on the damaged and those on the undamaged leaves.

The tissues adjacent to the point of inoculation darkened to an intense brown and this area was surrounded by an irregular ring, greyish yellow towards the centre with a well-marked margin (Plate XLI, fig. 4). The entire lesion was enclosed by a halo due to a faint but perceptible yellowing of the leaf. The lesions increased somewhat in size, but did not spread either to other parts of the leaf or to the healthy leaves. Four of these plants were allowed to continue growth. Forty-eight days after inoculation secondary infection was observed on the fifth leaf of one plant, and was followed by its appearance 2 days later on the fifth and sixth leaves of the remaining three plants. In no case was secondary infection found on the second, third and fourth leaves. The secondary lesions appeared as small red-brown areas scattered indefinitely on the leaf. Examination of the withered inoculated leaves and those showing the secondary stage revealed abundant spores, including the tri-pointed form. From these the fungus was easily re-isolated into pure culture. The controls throughout remained healthy.

Group II.

Spore inoculations.

Spores were obtained under the aseptic conditions previously described, and an emulsion was made in sterile water. The seedlings were grown in 12 in. pots as in the two previous experiments; eighteen plants were used, on nine of which the epidermis of the first leaf was ruptured. Drops of the spore emulsion were distributed over the first foliage leaves and the glass cylinders replaced. Within 48 hours lesions appeared on one leaf and in 4 days all the inoculated leaves showed well-developed lesions (Plate XLI, fig. 3). At the end of 10 days these infected leaves were dead, and the disease had spread to the second and third leaves. Viable spores were freely developed (Plate XLI, fig. 6), and the fungus was re-isolated from them. No distinction in respect of the severity of the attack could be made between the damaged and undamaged leaves. A marked feature of these inoculations was the rate at which the disease developed from the spores as compared with the mycelium, especially as the spore load of the emulsion was low. The controls again remained healthy. Details of these inoculations are given in Table III.

Table III.

Inoculum	Region of inoculation		No. of plants inoculated	No. of infections	Degree of infection
Mycelium	Coleoptile	(ruptured)	6	6	Slight
,,	,,	(not ruptured)	6	4	,,
,,	1st leaf	(ruptured)	6	5	Definite
,,	,,	(not ruptured)	6	6	,,
Spores	,,	(ruptured)	9	9	Extensive
,,	,,	(not ruptured)	9	9	,,

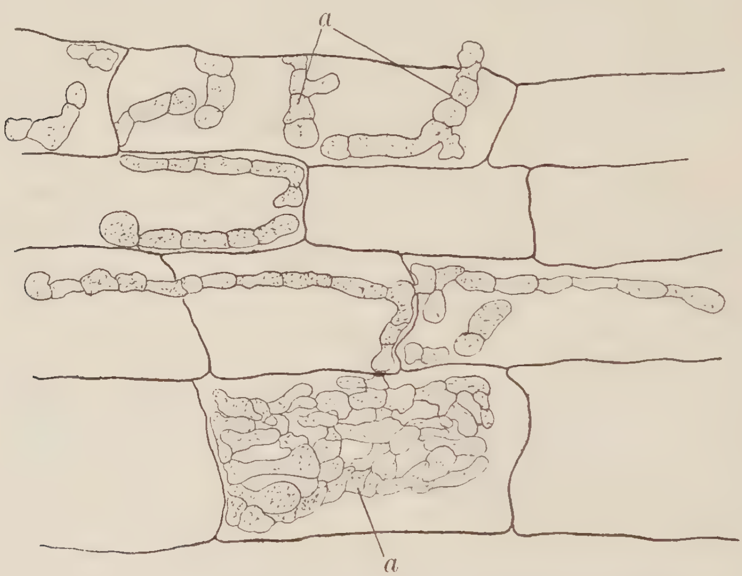
Cross inoculations.

Cross inoculations to wheat (var. Red Standard) and to barley (var. Spratt Archer) were made in order to ascertain whether the culture was pathogenic to these hosts. Sterilised seeds of each variety were sown in three groups of six in 12 in. pots as in the previous experiments. In the first series the plants were inoculated with mycelium, in the second with a spore emulsion, and the third remained as a control.

In all the cases where mycelium was used there was vigorous growth at first under the humid conditions of the tubes, but no lesions were formed. At the end of 48 days there was no indication of infection on any of the inoculated plants. The fungus is thus apparently not pathogenic to wheat or barley.

Control measures.

During the course of this investigation a number of trials have been carried out with the object of finding a means whereby the mycelium within the seed could be killed without damage to the embryo. The methods used included dressings of formalin at $\frac{1}{4}$, $\frac{1}{6}$ and $\frac{1}{8}$ per cent. strengths for varying time periods, dressings of 3 and 5 per cent. iodine dusts (3 and 5 per cent. iodine in diatomaceous earth) (12), and dry heatings (1) for 30 hours at 60, 80 and 100° C. respectively. All these treatments gave a decrease in percentage infection over the controls,



Text-fig. 3. Infected cells of the coleoptile. (*a*) Intracellular mycelium. Drawn under the camera lucida (× 600).

the most marked results being obtained with the stronger formalin dressings where the number of infected seedlings was reduced from 4·35 to 1·2 per cent.

It was our intention to carry out further trials on these lines, but the excellent results recently obtained by O'Brien and Prentice (7) in their extensive work on the control of the disease by Ceresan dressings suggest that this is a much more effective method. We are indebted to these workers for cultures of their *H. avenae sativae*, which on comparison appears to be identical with our own.

Morphological characters.

Mycelium.

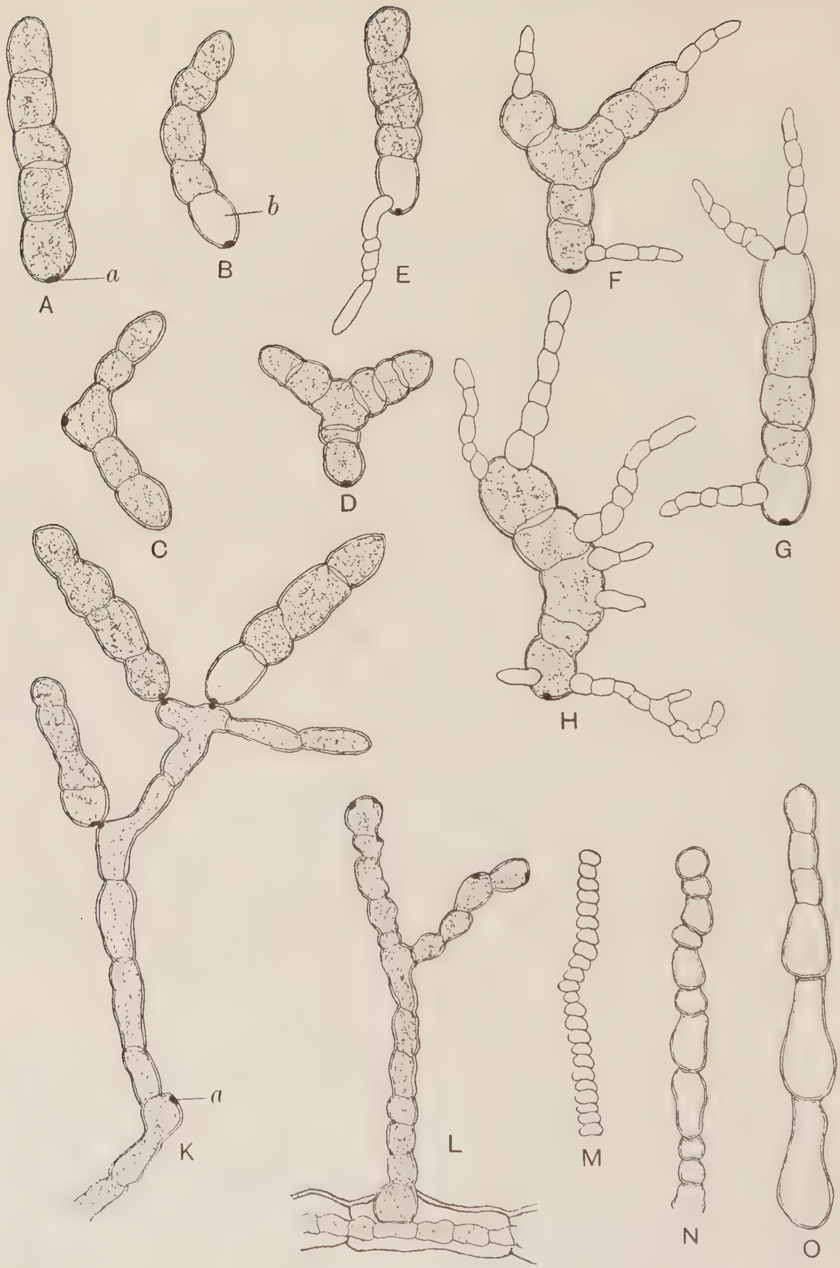
Two types of mycelium are developed, an aerial and a submerged form, both of which show certain marked differences and vary according to the substratum and condition of growth. These two types appear both in artificial culture and in natural infections. When cultured on media with high carbohydrate content or in the case of natural infection, when growing under moist conditions, the aerial mycelium becomes conspicuously dense, white and fluffy, and aggregates with erect conical arborescent tufts. The hyphae are hyaline, much branched and septate with extensive vacuolation.

The submerged form develops from 24 hours to several days later than the aerial, and is dark olive-green to black in colour with much modification in the cells. It is responsible for the zonation which is characteristic on certain media. In some cases it appears to be aggregated into small scattered areas, suggesting the development of sclerotia or perithecia, but no definite structure has been found. The hyphae break up easily and are much branched and septate. The cells are dark, thick walled and granulated. Many forms of hyphae are produced which range from long chains of small, unvacuolated, almost spherical cells which develop on potato-plug cultures to the long, vacuolated, club-shaped types found on synthetic media (Text-fig. 4 M, N, O).

The mycelium within the host cells (Text-fig. 3), especially in the palea or pericarp, approximates very closely to this submerged form. It is irregular, short, broad, highly septate, dark walled and much branched. Many of the cells, especially the terminal ones, are spherical; the contents are finely granular and show little vacuolation.

Conidiophores and conidia.

The conidiophores arise as solitary olive-green structures, occasionally in pairs but never in tufts (Text-fig. 4 K, L). They vary in length with age, and with the number of conidia produced, averaging 200–400μ in length and 8–9μ in width. The basal cell is slightly expanded, and in certain cases branching may occur. Emergence is most frequent between the cells of the epidermis and occasionally through the stomates. The conidia are borne terminally, but after the production of a spore the conidiophore elongates, develops one to three cells and again produces a terminal spore. This causes the typical "kneed" appearance, often



Text-fig. 4. A, basic spore form showing hilum at (*a*). B, C, D, variants of spore form showing clear basal cell (*b*) on B. E, F, G, H, germinating spores. K, conidiophore (*a*) point of attachment of first spore. L, branched conidiophore. M, N, O, hyphae from cultures: M, on potato plugs; N, on tyrosinate agar; O, on calcium malate agar. A–H, drawn under the camera lucida (× 600).

so pronounced as to form right-angled bends. The cells bearing the conidia are rounded and a scar marks the point of insertion.

The spore length varies considerably between 55 and 151μ with an average of 86μ; the width, whilst averaging 16μ, changes within the spore itself within the limits of 13–18μ. The basic spore form is straight cylindrical with more or less parallel sides tapering slightly at the terminal cell, and showing some restriction above the basal cell so that a club form is produced; the basal cell is hemispherical with a well-marked hilum (Text-fig. 4 A). There are many modifications of this form and this variation carried to its greatest limit appears as a forking at the second or third cell, producing a tri-pointed spore (Text-fig. 4 C, D). Stevens (15) reports this form as occurring frequently in a reputed culture of *H. teres* ex barley and in his own culture of *H. sativum* on rich carbohydrate agar; the form was lost, however, when transferred to corn-meal agar. We are not aware that it has been previously recorded for *H. avenae*, but we have found it regularly in the oat lesions both from natural and artificial infections. The length of each arm varies, but the typical ellipsoidal septa are developed in each. Between these two limits there is a wide diversity of forms with varying degrees of curvature and constriction.

The spores are sub-hyaline in their early stages, darkening on maturity to olivaceous green. The colour may be uniform over the whole except at the darker hilum, but in some mature spores the basal and/or terminal cells are sub-hyaline and devoid of granular contents (Text-fig. 4 B). No germ pores are visible. The maximum size is reached before septation, a feature reported also by Ravn and Stevens. The number of septa varies from one to seven, the latter number being quite frequent. Drechsler states that "whilst the number of septa in the spores of the stripe fungus (*H. gramineum*) very rarely exceeds seven, in the species *H. teres* and *H. avenae* eight and nine cross walls may be found readily and even ten or eleven occur in a small proportion of instances."

With our culture of *H. avenae* this is certainly not the case, since neither in natural nor artificial infections have any spores been found at any time with more than seven septa.

Table IV gives an analysis of 127 spores examined, taken from a natural disease lesion.

The septa appear ellipsoidal, confirming the cylindrical nature of the spore, and most frequently they lie parallel to one another except in the more abnormal spores, but even in these cases they are never sufficiently exaggerated as to produce muriform walls. The small circular

pits or spots on the cross walls referred to by Drechsler in *H. teres* and *H. giganteum* are apparent in plasmolysed but not in living spores. No secondary spores, such as develop in *H. sativum*, have been found.

Table IV.

No. of spores	No. of septa per spore	%
4	2 septate	3·1
9	3 „	7·0
13	4 „	10·2
37	5 „	29·8
41	6 „	31·2
23	7 „	18·1

Germination takes place within 90 minutes in tap-water at 24° C., and is both terminal and intermediate (Text-fig. 4 E, F, G, H). The optimum temperature lies between 20 and 24° C. Germination is retarded at 30 and 15° C., practically ceases at 37° C., and at 10° C. is much delayed, subsequent growth being slow. The spores are killed when immersed in water at 50° C. for 5 minutes and at 45° C. for 10 minutes.

Discussion.

The results obtained enable us to reach some conclusions in respect of the relationship of leaf-spot and its causative organism with the spotting and striping diseases of oats described by earlier workers, and for which *H. avenae*, *H. gramineum* and *H. teres* have all been cited respectively.

Comparing first such data as is known of the characters of *H. avenae* with those of our organism we find close, though not absolute, agreement.

Thus, Ravn's culture of *H. avenae* produced no spores in culture on artificial media, whilst Stevens' culture produced spores only on sterilised wheat straw—a close parallel with the behaviour of our own culture. Again, our organism agrees with Drechsler's in the tendency towards branching of the conidiophore, the basic spore form and the mode of spore germination, but differs from it in the length of the conidiophores and the maximum number of septa in the spores.

In respect of the external symptoms of the disease our observations tally closely with Eidam's descriptions of the primary stage so far as this goes—the non-pathogenicity of his culture to wheat and barley is also a character of our own.

Our record of two distinct phases of the disease is confirmatory of that noted by Ravn and Drechsler. There thus seems little doubt that

the disease, as we know it, is the same as that described by Eidam and possibly as that of Briosi and Cavara, and that our organism is the *H. avenae* (Bri. and Cav.) Eid. Both Ravn and Drechsler are inclined, on what seems to us somewhat slender evidence, to regard this fungus as a biological species of *H. teres*. The two fungi are compared in Table V, taking the characters of our own organism as representative of those of *H. avenae*.

It will be seen from the above that whilst the morphological differences between the two fungi are comparatively slight they are definite, and that the fungi behave differently in artificial cultures. It seems to us

Table V.

	H. teres	Own culture
Conidiophores	$120–200\mu \times 7–9\mu$ rarely branching (Drechsler)	$200–400\mu \times 8–9\mu$ with some branching
Spores		
No. of septa	1–10	1–7
Length	$30–175\mu$	$55–151\mu$
Breadth	$15–22\mu$	$13–18\mu$
Shape	Basic form cylindrical. Stevens once reported the tri-pointed form on a reputed culture of *H. teres*. Not seen by present writers	Basic form cylindrical with a variant tri-pointed form common under natural and artificial infection
Colour	Uniform. Sub-hyaline to light fuliginous at maturity	Sub-hyaline to light fuliginous at maturity with the basal and/or terminal cells frequently colourless
Sporulation in culture	Spores readily on tap-water and Beijerinck's agar with production of secondary spores, and on sterile leaves and beer wort and barley leaf decoction agars	Does not spore on these media. Spores very sparingly on sterile oat leaves
Pathogenic to	Barley but not to oats	Oats but not to barley or wheat

undesirable to force what is undoubtedly a close relationship into an identity. Moreover, we would point out that in biological species the symptoms of disease in the different hosts are closely similar. This is far from being true in respect of the leaf-spot of oats, and forms an additional argument for retaining separate specific rank for the respective organisms.

A a few words must be said in regard to *H. gramineum*. This fungus has not been found by the writers in any of the spots or stripes associated with this oat disease, and we cannot, therefore, confirm the records of Ritzema Bos, or Johnson (4). It has already been pointed out that stripe lesions frequently occur (confirmatory of Smith), but

that these stripes originate from the coalescence of spot infections. Subsequent to the conclusion of this investigation we have had the pleasure of discussing the problem with Mr O'Brien of the West of Scotland Agricultural College, Glasgow, who abundantly confirms our record of striping and actually calls the disease leaf-stripe. This is obviously the most descriptive name for the disease in its most virile form.

As regards the mode of attack we are unable to confirm Ravn's suggestion of a strong analogy between leaf-spot and the cereal smuts in their manner of infection, since in leaf-spot the invading mycelium only reaches the growing point in the final stages of the disease. Moreover, we find no parallel with the attack of *H. gramineum* on barley as described by Smith. The latter, in fact, would be almost precluded by the existence of the two well-marked phases of the leaf-spot disease.

Summary.

1. The symptoms of the disease are defined. There are two well-marked phases as described by Ravn (1) on the seedling, (2) on the mature plant. The first of these is responsible for considerable loss in seedling oats. The second brings about the infection of the grain. Both spot and "stripe" lesions are produced.

2. The disease is especially dependent upon climatic factors. Under adverse conditions the extent and degree of infection is greatly increased, whilst with favourable conditions even plants from infected seed may escape the disease. It is for this reason that the disease assumes serious proportions in northern districts, where an inclement spring is the rule rather than the exception.

3. The causative organism of the disease has been isolated and its pathogenicity verified. It is not pathogenic to wheat or barley.

4. The disease is seed borne by adherent spores and resting mycelium within the pericarp. The percentage infection by spores on a number of commercial samples of Scottish grown oats ranged from 6 to 62 per cent.; the resting mycelium, however, is the more common and the more dangerous type of infection.

5. The infection of the seedling by the fungus has been investigated. The mycelium within the pericarp penetrates the scutellum and the epiblast during the early stages of germination, and this is followed by the invasion of the first, second and third leaves. If the growing point

is reached the seedling is killed. Spores are produced abundantly on the dead leaves and initiate the secondary stage on the fifth and subsequent leaves.

No parallel has been found between the mode of infection described and that stated to occur in the smuts. The production of a "stripe" is due to the coalescence of individual spots, and there is, therefore, no analogy between the formation of such lesions and those of stripe on barley as described by Smith.

6. The morphological and cultural characters of the causative fungus have been described and compared with those of other workers. The conclusion is drawn that it is identical with the *H. avenae* (Bri. and Cav.) Eid., and that whilst its close relationship to *H. teres* is admitted there are certain morphological and considerable cultural differences between the two species.

7. Control measures for the disease are discussed.

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EXPLANATION OF PLATES XL—XLI.

PLATE XL.

Fig. 1. Natural primary infection of the coleoptile and first leaf of oat seedlings, showing the characteristic distortions (photographed from a painting).

Fig. 2. Natural primary infection on oat seedling. The first and second leaves have been killed and a "stripe" lesion is seen on the third leaf.

PLATE XLI.

Fig. 3. Oat leaves inoculated with spores of *H. avenae* after 4 days.

Fig. 4. Oat leaves inoculated with mycelium of *H. avenae* after 8 days.

Fig. 5. Natural secondary infection of the upper leaves of oats.

Fig. 6. Microphotograph of mycelium, conidiophores and spores of *H. avenae* produced on the leaves in Fig. 3 after 7 days.

(*Received February* 10*th*, 1931.)

Fig. 2

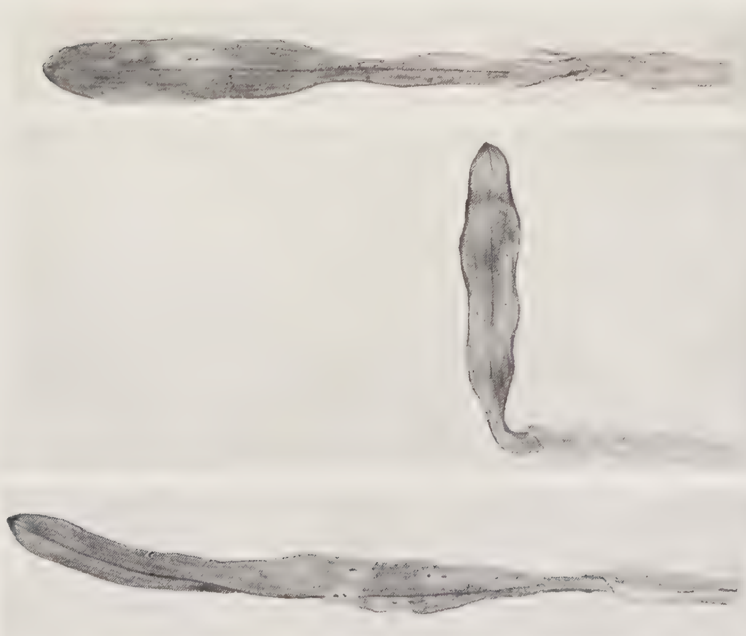

Fig. 1

TURNER AND MILLARD.—LEAF-SPOT OF OATS *HELMINTHOSPORIUM AVENAE* (BRI. AND CAV.) EID.
(pp. 535–558).

Fig. 3.

Fig. 4.

Fig. 5.

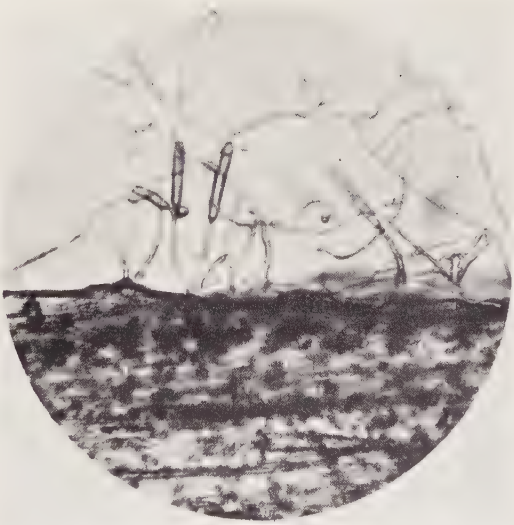

Fig. 6.

TURNER AND MILLARD.—LEAF-SPOT OF OATS *HELMINTHOSPORIUM AVENAE* (BRI. AND CAV.) EID.
(pp. 535–558).

THE ABSORPTION OF WATER BY SEEDS OF *LOLIUM PERENNE* (L.) AND CERTAIN OTHER GRAMINEAE

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(With 8 Text-figures.)

In the course of some investigations on the germination rates of different types of perennial rye grass seeds it was found necessary to examine the water absorption mechanism of the typical seed. Investigations were designed to determine:

(1) The effect of the pale and of the seed coat on the absorption of water.

(2) The extent to which the absorption of water is restricted to certain areas of the seed coat.

(3) The causes of such localisation.

The investigations were carried out on perennial rye grass seeds; seeds of wheat and barley were also used for comparative purposes.

Method.

Two complementary methods were used in these investigations:

(1) The direct method of weighing after immersion in water.

(2) The iodine method.

By the method of direct weighing the rate of absorption by the seed can be determined fairly accurately. The seeds are placed in small wire cages and then immersed in water. After removal from the water and the cages the seeds are pressed between filter paper and then weighed. Figures obtained by this technique represent total absorption. During the course of these investigations a modification has been developed, by means of which determinations can be made of the relative rate over different parts of the seed.

Floats of about an inch square are prepared from waxed filter paper. In the centre of these an orifice is burnt out, just large enough to allow the seed to be drawn through it to the extent of about an eighth of an inch. The seed, after removal of the pales, is sealed into the orifice with

a mixture of paraffin wax and vaseline. When the float is on the water absorption is confined to a restricted area of the seed coat. Weighing can be done with the seed still attached to the float, or after separation from the float. If repeated weighings have to be made on the same sample, the seeds are not separated from the floats, but after removal from the water the lower surfaces of the floats and of the seeds are dried and then weighed.

The most satisfactory seal between the float and the seed is obtained with a mixture of 30 per cent. vaseline, and 70 per cent. paraffin wax, the paraffin wax having a melting point of 105° F. The efficiency of the seal as a barrier to the upward spread of the water over the outer surface of the seed was tested by applying cobalt chloride paper to the exposed surfaces of the seed above the float. No change was noted in the colour of cobalt chloride paper applied to seeds that had been under test for 2 hours.

Seeds immersed in a solution of iodine and potassium iodide in water become discoloured in the areas into which the iodine has penetrated. Iodine penetration has been accepted by some investigators as evidence of the penetration of water. The test has, therefore, been used as an indicator of the areas of localised water absorption and of the rate and extent of the diffusion of water in the endosperm.

The sequence of iodine penetration seems to follow that of water. The areas of earliest endosperm discoloration are also those of highest water content. It therefore seems justifiable to assume that the iodine test does give some indication of comparative rates between different parts of the same seed, or between different seeds. It cannot, however, be used for the determination of absolute rates.

Influence of pales on the absorption of water.

The influence of the pale was examined (1) by determinations of total absorption by seeds with their pales, and others without pales, and (2) by observations on the behaviour of pales, when the seeds are totally immersed, and when the bases of the seeds only are immersed.

The rate of total absorption is depressed slightly by the removal of the pales. The greater initial absorption of seeds with their pales may be due to the water absorbed by the pales themselves (Fig. 1).

When immersed completely in water the attachment between pale and pericarp is quickly broken. The separation commences at the base of the seed; within half an hour it reaches the middle of the seed, and within 2 hours the separation is complete. This separation, it would seem,

is necessary before water can reach the general surface of the pericarp. The outer surface of the pale is only very slightly permeable to water. Pales were sealed over the end of a glass tube filled with water, and with their normally exposed surfaces in contact with the water. Water only appeared on the lower surface of the pale 2 hours after the commencement of the experiment. Moreover, half an hour after immersion the area of the seed coat detached from the pale is wet, whereas the surface of the upper half of the seed coat that is still attached to the pale is dry.

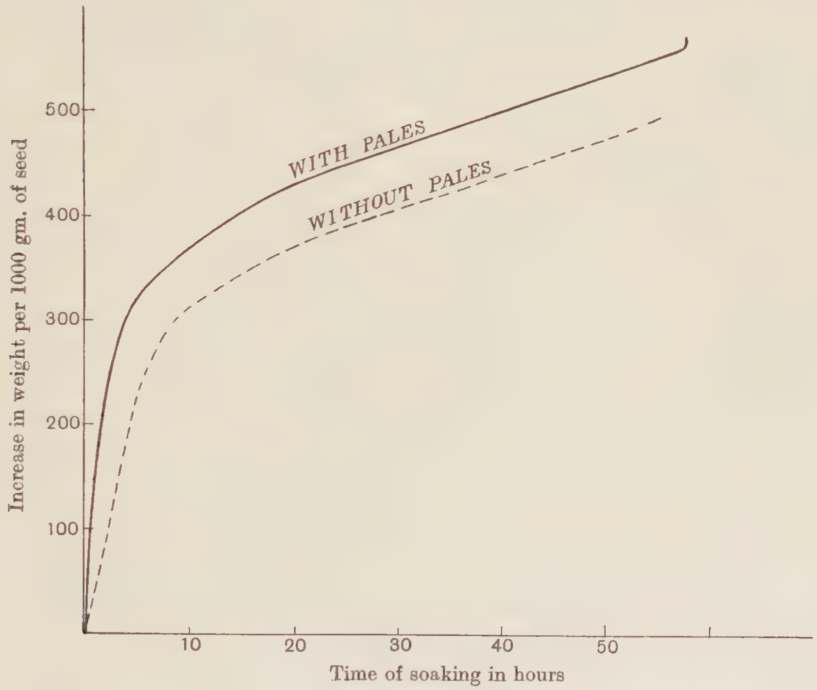

Fig. 1.

The separation of pale and pericarp is the result, it seems, of swelling in the grain. The seed coat is stretched, but the pale does not stretch correspondingly. The breadths of pales were measured before and after soaking in water, and no increase was noted after immersion. Thus, the attachment is broken by a shearing effect. Evidence will be adduced in a later section, to show that the area of absorption is at first limited to the base of the seed, and later spreads progressively upwards. Swelling, therefore, also commences at the base and spreads upwards. Hence, the

earlier separation of pale and pericarp at the base, and its later extension to the upper parts of the grain.

Collins (1) in barley found that the pales assist the absorption of water to the extent of distributing water very rapidly over the surface of the pericarp. No such distribution was noted in rye grass. Before the separation of the pale from the seed, water reaches the surface of the pericarp only along two limited longitudinal tracts:

(1) The edges of the grain on the ventral surface of the seed.

(2) The sides of the seed.

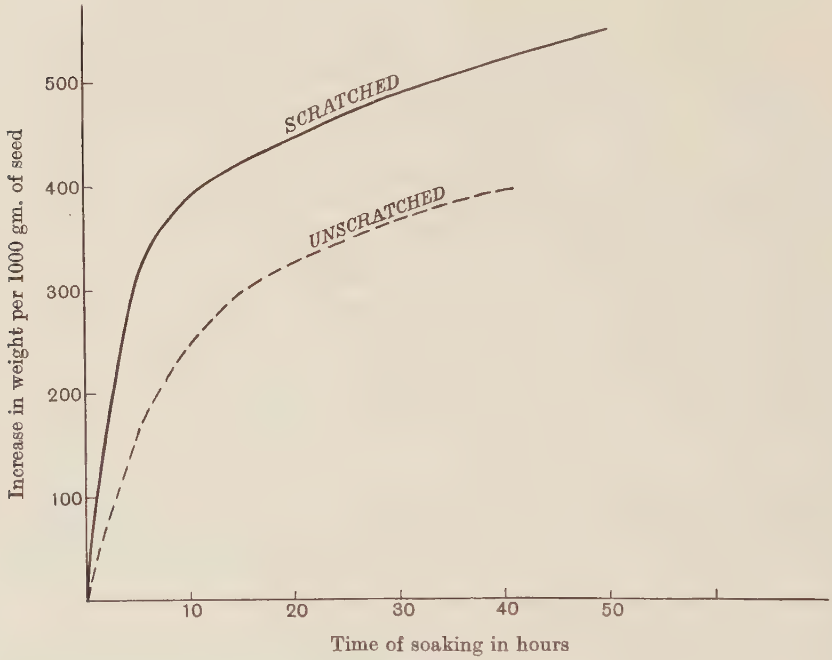

Fig. 2.

The distribution of water over these areas is apparent in seeds in floats with their germinal ends immersed in water, or better in an aqueous dye solution. The dye solution rises to the apex of the seed within 15 minutes after the seed is floated on the solution. There is no lateral extension of the reddened area before the swelling of the seed. At the edges of the grain on the ventral surface the pale is raised away from the pericarp; a closed channel is thus produced from the base to the apex of the seed. Water rises along this channel as soon as it has gained access to the pericarp at the base (Fig. 3).

At the sides of the seed the pales overlap, and here they are not attached to each other, nor is the inner one attached to the pericarp. Thus, water has direct access to the pericarp in this region of the grain. The upward movement of water at the sides is probably helped by furrows that are formed in the pericarp of this region (Fig. 4).

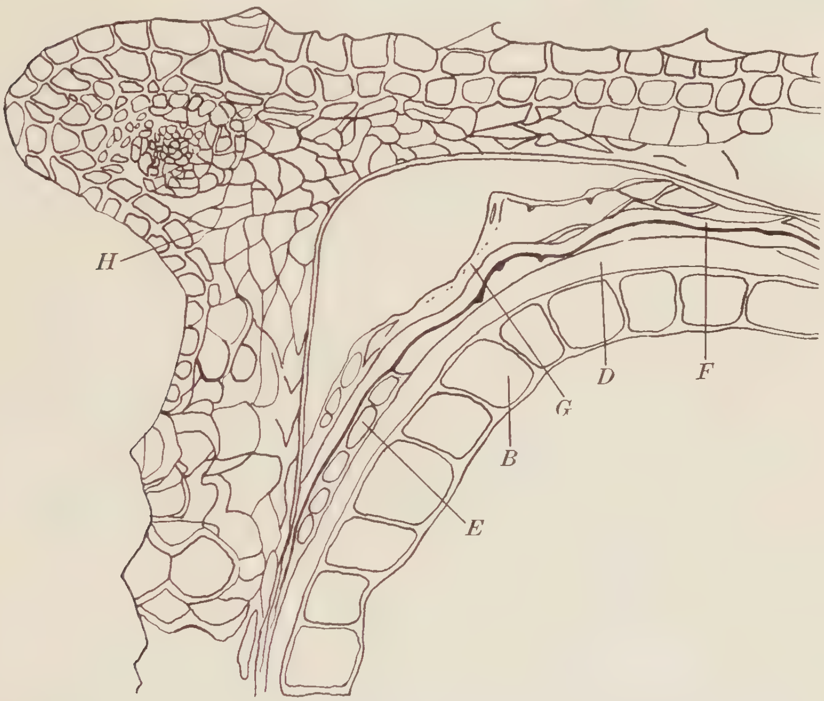

Fig. 3. Edge of ventral surface. *B*, aleurone layer; *D*, hyaline layer; *E*, testa; *F*, cross layer; *G*, outer epidermis of pericarp; *H*, pale.

It is interesting to note that in seeds in which the barriers to the penetration of water in the seed coat are destroyed, penetration of water at the sides and edges takes place simultaneously with penetration at the base. For reasons to be noted later it can be assumed that in this case the limited absorption above the base has taken place before the swelling of the seed. The attachment of pale to pericarp has not been broken, and, therefore, absorption has only been possible over the limited areas not covered by the pales.

Influence of seed coat on the absorption of water.

As anticipated the seed coat was found to exercise a retarding effect on the absorption of water. The absorption of seeds which had had their seed coats scratched was compared with that of seeds with intact coats. The results are presented in graphical form in Fig. 2.

Collins found that the barriers to the penetration of water in the seed coat of barley are certain membranes that he identified as cuticular. Observations on rye grass indicate that the corresponding membranes also retard the absorption of water. Direct evidence to this effect could not be obtained. The assumption was made that because these membranes stopped the passage of substances in solution they would retard

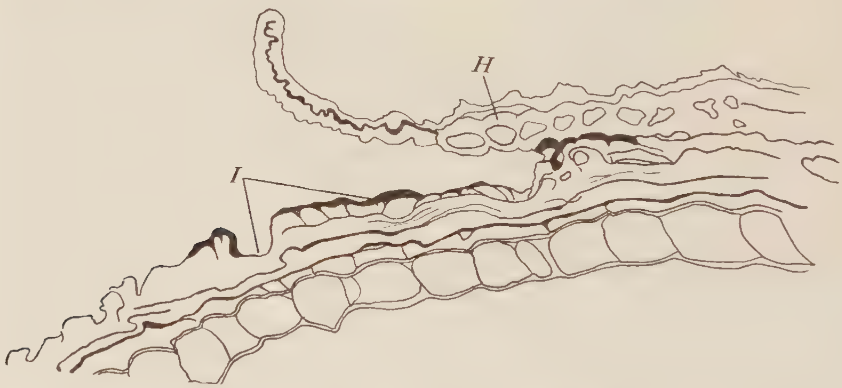

Fig. 4. Side of the grain; *H*, inner pale; *I*, corrugations of seed coat.

the diffusion of water across the seed coat. Seeds were immersed in aqueous dye solutions and in solutions of silver nitrate. In all cases the solute penetrated only as far as a membrane that is attached to the outer surface of the testa. These membranes give the reactions of cuticle, but at the same time they give some of the microchemical reactions of true fats. They give the reactions of cuticle with (1) alcoholic chlorophyll solution, (2) alkanna, (3) Kroemer's Sudan III. The two latter, of course, give the same reactions with either chlorophyll or fat. In the case of true cuticle, however, Kroemer's Sudan III will not turn the membrane red until it has been heated, but some of these membranes turned red immediately the reagent was added. Moreover, the membranes nearly always turned black with osmic acid—a reaction that is never given by cuticle of foliage leaves.

The cuticle of the foliage leaf and the cuticle-like membranes of the seed also seem to differ in their physical properties. The elasticity of the latter seems to be at least five times greater than is that of the former.

Priestley (2) suggests that cuticle is an aggregate containing the anhydrides of various cutinogenic acids, together with the glycerides of these acids, or true fats. The microchemical tests detailed above suggest that the so-called cuticle of the seed coats of grasses contains a higher proportion of true fat, with a correspondingly lower content of the anhydride. According to Priestley the impermeability of cuticle

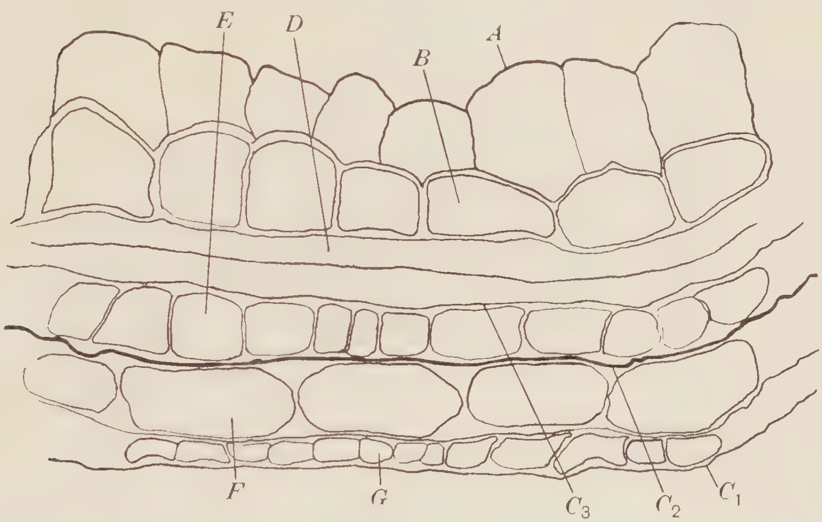

Fig. 5. T.S. of seed coat on dorsal surface. *A*, endosperm cells; *B*, aleurone layer; C_1, cuticle of outer pericarp epidermis; C_2, outer cuticle of testa; C_3, inner cuticle of testa; *D*, hyaline layer; *E*, testa; *F*, cross layer; *G*, outer epidermis of pericarp.

for water is due to the anhydride. True fats, on the other hand, are permeable to water (Kahlenberg (3)); the cuticle-like membranes of the seed coat might, therefore, be expected to be more permeable to water than is normal cuticle.

In the sequel the term cuticle is used where cuticle-like membranes are implied, in the sense indicated above.

Cuticular membranes were noted in the following positions:

(1) On the surface of the outer pericarp epidermis.

(2) On the outer surface of the testa.

(3) On the inner surface of the testa.

The three membranes differ considerably in thickness. The thinnest is that on the outer surface of the pericarp, the thickest is that on the outer surface of the testa (Fig. 5).

The cuticular membranes are interrupted only by a corky strand in the furrow of the ventral surface (Fig. 8). At the base of the grain opposite the micropyle the cuticular membranes are not as thick as they are elsewhere. Collins reports an absence of cuticle opposite the micropyle of barley. Netolitzky (4), however, states that these membranes are

Fig. 6. Base of seed, C_2, outer cuticle of testa.

present in the tegumentary layers opposite the micropyle. The present writer also found cuticle across the micropyles of seeds of wheat, barley and rye grass (Fig. 6).

Paths of absorption.

The extreme thinness of the cuticular membranes immediately opposite the micropyle points to this as the area of earliest absorption. Such was found to be the case on seeds soaked in iodine solution for an hour. In all seeds early basal penetration was noted.

In certain exceptional seeds in addition to early basal penetration, absorption had taken place over one of two other areas as well, (1) at the apex of the seed, (2) over the general surface of the seed coat.

Apical penetration is confined to seeds in which a caruncle-like structure is developed at the apex. The outer epidermis of the pericarp is lifted some considerable distance above the testa, and the intervening space is occupied by large thin-walled parenchymatous cells. This tissue absorbs dye solutions readily (Fig. 7).

Seeds in which early penetration of the seed coat had taken place were always heavily infected in the seed coat with an endophytic fungus. At intervals on the outer surface of the pericarp the fungus seemed to be forming pycnidia-like bodies, and on no other seeds were these observed. The identity of the fungus has not been determined; it is, however, probably related to the mycorrhizal fungus on the roots

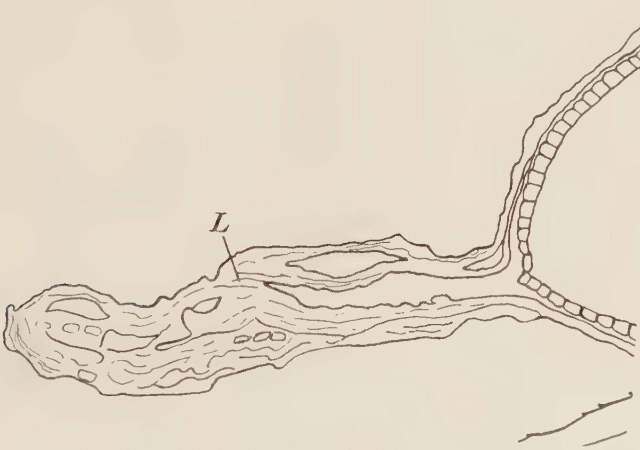

Fig. 7. Apex of seed. *L*, Caruncle-like tissue.

(McLennan (5, 6)). The greater permeability of seed coats infected by this fungus is probably due to extensive piercing by the hyphae. Sections of these seeds were examined in the earliest stages of iodine absorption, and it was found that iodine appeared first in the endosperm at the sides of the seed. The dorsal and ventral surfaces of the grain are covered by the pales, and until the pericarp and the pale are separated by the swelling of the seed, iodine cannot reach the pericarp in these areas. Absorption, therefore, only takes place at the sides, which are not covered by the pales, and which are in direct contact with the solution.

Early basal absorption takes place in all seeds, and in most seeds early absorption is only basal. In iodine-soaked seeds the iodine appears in the endosperm on the shoulders of the furrow on the ventral surface 15 minutes after immersion. Initially the iodine-stained area does not

spread upwards on the ventral surface. From the base of the ventral surface it moves diagonally across the grain to the dorsal surface over the scutellum. Blueing then spreads towards the apex of the seed in the small endosperm cells immediately beneath the aleurone layer of the dorsal surface. The upward spread on the ventral surface only commences when the lower half of the dorsal surface has been stained. Collins has noted a similar sequence in barley.

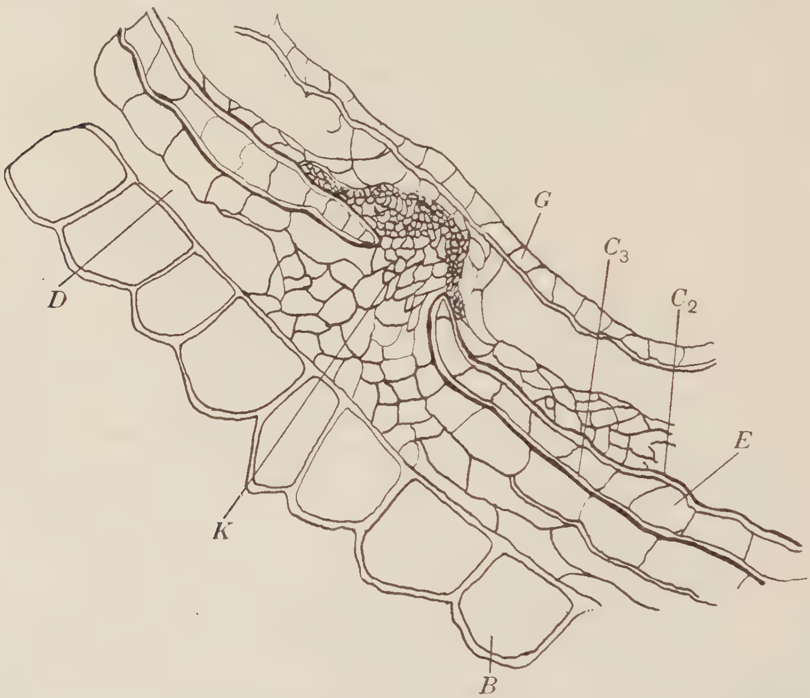

Fig. 8. Furrow on ventral surface of seed. *B*, aleurone layer; C_2, outer testa epidermis; C_3, inner testa epidermis; *D*, hyaline layer; *E*, testa; *G*, outer pericarp epidermis; *K*, cork strand.

Two entirely different interpretations have been put on the results of iodine immersion tests.

Collins suggests that iodine, and by inference water also, are absorbed only at the base of the seed, and that they spread upwards by diffusion; absorption through the general surface of the seed coat being exceptional. His interpretation involves two assumptions, (1) that the seed coat above the micropyle is impermeable to water, and (2) that diffusion of both water and iodine takes place in the endosperm.

Evidence is adduced elsewhere that suggests that the cuticular membranes that Collins suggests are impermeable to water are probably a good deal more permeable to it than is, say, the cuticle of the leaf of cherry laurel.

Shull (7) and Beeskow (8) have shown that there is no sub-aleuronic diffusion of iodine. Experiments were made by the present writer to determine the extent of the upward diffusion of water. Seeds were placed on floats with the bases of the seeds just immersed in the water. Twelve hours later the seeds were removed from the water, and separated from the floats; they were then divided into basal, apical and middle portions, and the water content of each was determined. These figures were compared with the water content of corresponding portions of the air-dry seeds. It will be seen from the figures of Table I, that diffusion of water in the endosperm is very slight; a slight amount, however, does occur. Collins' assumption of diffusion from base to apex is therefore untenable. Moreover, if absorption is confined to the base, seeds totally immersed and seeds on floats with their germinal ends only immersed should absorb water at approximately the same rate. Absorption rates of the two cases are compared in Table II, and it will be noticed that seeds totally immersed absorb water very much more rapidly than do seeds with only the germinal ends immersed.

Table I.

Percentages water content of basal, apical, and middle portions of the seed (A) before, (B) after basal absorption for 12 hours.

	(B)	(A)	(B)	(A)	(B)	(A)
Apical	14	12	16	12	13	12
Middle	17	12	18	12	17	12
Basal	20	12	18	12	21	12

Table II.

Water absorbed per 1000 *gm. of seed totally immersed and seeds with bases only immersed.*

Time in hr.	Total	Basal
1	150	55
2	215	75
3	275	105
6	290	110
9	320	115
2	350	120

Shull has attempted to explain the results of iodine immersion tests on the basis of Schroeder's theory (9) of a progressive increase in the

permeability of the seed coat to water from apex to base. As the chief barriers to the penetration of water and of iodine are the cuticular membranes, Schroeder's theory would imply either a decreasing thickness of cuticle from apex to base, or a change in the composition of the cuticle corresponding to the increase or decrease of permeability. Measurements of the cuticular membranes have been made, and there appears to be no difference between the thickness of membranes at the apex and those immediately above the embryo at the base. It is shown below that the normal sequence of absorption can be reversed, and absorption induced in the upper parts of the seed before it has taken place immediately above the embryo, by increasing the water content of the upper part of the seed relative to that of the lower. These results seem to indicate that the differences in the rates of absorption over different parts of the seed are determined by variations in the internal water content, and not by variations in the permeability of the seed coat to water.

The very slight diffusion of iodine in the endosperm and the progressive extension of the iodine-stained area from base to apex when the seeds are immersed in the iodine solution, indicate that the area of absorption is at first confined to the base, and later spreads upwards. But as suggested in the last paragraph it seems probable that there is uniform permeability in the seed coat of the dry seed from a point just above the embryo to the apex. Thus, it is necessary to assume that during absorption there is some agent inducing increased permeability and that this agent acts progressively from base to apex.

Experiments made by the present investigator show that the permeability of the seed coat to water depends upon the amount of water already present in the endosperm. Seeds were floated on water with the bases of the seeds immersed. The seeds were then inverted into iodine, and the time of the earliest appearance of iodine in the endosperm noted, and this was compared with the time of the earliest appearance of iodine in the endosperm of dry seeds attached to the float and with approximately the same portions of the seed in contact with the iodine solution. The average time in the first case was 5 minutes, in the second case 30 minutes. The above was repeated on seeds with the apex instead of the base immersed during the first 12 hours. When these were inverted into iodine the surface of the seed around the embryo was waxed. The time of the earliest appearance of the blue colour in the endosperm of dry seeds was again 30 minutes, in those that had been previously absorbing water it was approximately 10

minutes. The area of the earliest penetration of iodine in these seeds is of interest. It occurred immediately beneath the float at the point furthest away from the embryo. In this case the area immediately beneath the float is that of greatest water content, being nearest the point of previous absorption—the apex.

The absorption of water by the seed leads to swelling. The immediate effect of swelling is a stretching of the seed coat, and it seems probable that it is to this that the increased permeability of the cuticle is due. The increased permeability, however, is not the result of breakage consequent upon stretching. When seeds that have been soaked are allowed to dry the permeability of the seed coat is approximately of the same order as that of the normal case. The effects of stretching relevant to the permeability of a membrane are probably two, (1) a decrease in the thickness of the membrane, and (2) an increase in the size of the pores of the membrane. The decrease in thickness might be expected to increase the rate of diffusion of iodine and water across the membranes of the seed coat. Similarly an increase in the size of the intermolecular pores would admit a greater volume of water into the seed in unit time. (Sieve theory of membranes, Bayliss (10), Stiles (11).)

Experiments were made to test the effect of stretching on the permeability of certain membranes. In only one case was it found possible to demonstrate an increased permeability after stretching. The rate of diffusion of pyridine across a rubber membrane was increased three times by this means. In all other cases it was found impossible to stretch the membranes without damaging them.

Livingstone has suggested that it may be that the permeability of protoplasmic membranes is increased by stretching (12).

The stretching of the seed coat is the result of swelling of (1) the endosperm, and (2) of the testa. The testa is mucilaginous. In the dry seed the layer is 10μ thick, but on soaking in water it swells to a thickness of 30μ. When placed in alcohol the walls of the water-saturated testa shrink. When transferred back into water they swell again. Moreover, the layer gives the reactions of mucilage with corallin soda.

The water that enters at the micropyle is capable, as pointed out above, of a slight upward diffusion. The diffusion into the drier parts of the seed leads to swelling, and that, in the manner indicated above, induces a greater permeability of the seed coat. The water that enters as a result of the greater permeability at this higher level diffuses upwards, and in its turn induces greater permeability in the seed coat further up still.

The furrow on the ventral surface of the seed renders effective stretching of the seed coat in that area very slight. Pressures acting dorso-ventrally would tend to straighten the line of the ventral surface, and would not, therefore, stretch the seed coat on that side. On the dorsal surface any dorso-ventral pressures act directly on the seed coat, and therefore presumably induce more effective stretching. Hence the earlier appearance of the iodine under the aleurone layer of the dorsal surface. The suggestion advanced above is supported by the facts of iodine penetration at the apex of the seed. The furrow disappears some distance below the apex of the seed. In the upper part of the grain between the apex of the furrow and that of the seed the iodine appears uniformly within the aleurone layer.

The whole process of the progressive extension upwards of the area of absorption is started by the initial absorption at the base. This early basal absorption is the result of three conditions:

1. A high absorbing power of the base of the rachilla for water. When a drop of eosin is placed on this structure the red dye very rapidly spreads to the micropyle.

2. The extreme thinness of the cuticular membranes across the micropyle.

3. The high absorbing power of the embryo for water. No means was found of measuring this directly, but an indirect indication is possible by measuring the water contents of base, apex and middle portions of the seed during drying. Seeds were soaked in water for 4 days, removed and allowed to dry. The water content of base, apex and middle portions of the seed was determined on samples selected at intervals of 3 hours. At all stages of drying the water content of the base was greater than that of the other two parts.

Summary.

1. The methods used in these investigations into the problems of absorption of water by seeds are described. A modification in the method of direct weighing is suggested.

2. The rôle of the pale in absorption is discussed. The pale exercises a retarding effect on water absorption, so long as it is attached to the caryopsis. But the attachment is broken as the absorption of water with consequent swelling extends from base to apex.

3. The cuticular membranes of the seed retard the absorption of water, but the permeability is increased by the stretching of the cuticle, consequent upon swelling.

4. The earliest absorption takes place through the micropyle. This water can diffuse upwards to a certain extent. At the higher level it causes the endosperm to swell, which in its turn induces greater permeability of the cuticular membranes. The water absorbed at this higher level diffuses upwards to a slight extent, and causes greater permeability at a still higher level. Thus, there is a progressive extension upwards of the area of absorption induced by the slight upward diffusion of water.

The writer wishes to acknowledge his indebtedness to Prof. W. Stiles for help in the preparation of the manuscript of this paper; to Mr F. R. Horne for help in the course of the work; and to Mr R. L. Stevenson for making the diagrams which accompany this paper.

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THE BIOLOGY OF THYSANOPTERA WITH REFERENCE TO THE COTTON PLANT

VII. THE RELATION BETWEEN TEMPERATURE AND HUMIDITY AND THE LIFE CYCLE

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(With 3 Text-figures.)

During 1926 a series of experiments was made to find the effect of atmospheric humidity on the development of *Thrips tabaci.* In these experiments sulphuric acid of varying strengths was used to maintain a constant degree of relative humidity, but for several reasons the methods used were found to give unsatisfactory results. In the first place, fairly large vessels were used to contain the acid and cotton leaves with the insects were suspended inside them so that, in addition to the numbers of thrips that were killed by falling into the acid, it is possible that many of the insects were overlooked when they were being counted owing to their small size and the fact that they were contained in comparatively large jars; also it was impossible, except by constantly changing the acid, to be certain that the relative humidity in the jars remained even approximately constant for any length of time; lastly, the experiments were carried on in the laboratory and no attempt was made to control the temperature. It did appear, however, that at temperatures varying between 10 and 20° C. a high degree of atmospheric humidity, contrary to the generally expressed view, is not a factor which affects thrips larvae adversely; so that it was decided to repeat the experiments in a more exact manner.

Technique.

In the experiments described in the following paper, the temperature was controlled by means of a multiple temperature incubator (5) and the degree of relative humidity by a series of saturated solutions of certain salts (1, 3); these solutions have the great advantage that while a little of the undissolved salt remains they will automatically maintain themselves in a saturated condition even if more water is taken up.

Glass tubes, $1\frac{1}{2}$ in. by 4 in., containing about $\frac{1}{2}$ in. of the solution were used, and the larval thrips and pieces of cotton leaf were placed in smaller tubes closed by bolting silk, so that there was no danger of the larvae escaping into the solution in the larger tubes. Various salts were tried, and finally four substances were chosen which gave a wide range of relative humidity which remained fairly constant at the temperatures used in the experiments.

The salts used were:

Sodium sulphate	giving	a relative humidity	of	80–85	per cent.
Sodium chloride	,,	,,	,,	73–76	,,
Cupric chloride	,,	,,	,,	66–68	,,
Magnesium chloride	,,	,,	,,	29–32	,,

In addition, water was used to give a saturated atmosphere.

The humidities referred to in the following experiments are not absolutely exact, as it is impossible to keep the degree of relative humidity quite constant when dealing with insects which have to be examined daily and are fed on fresh leaves, but it is held that the humidity is approximately indicated by the figures and that the experiments give a definite indication of the effect of atmospheric humidity on the life cycle of *T. tabaci*.

INFLUENCE OF HUMIDITY ON MORTALITY.

In the first place, the effect of varying relative humidity on the rate of mortality of the insect at different temperatures was considered, and the number of larvae reaching the prepupal, pupal and adult stages was counted. The following table gives the percentage of larvae reaching the further stages in development at the different relative humidities:

Relative humidity (%)	No. of larvae	Stage reached (%) Prepupal	Pupal	Imaginal
100	3600	14·2	11·1	6·8
85	2600	17·7	14·9	10·9
75	5100	19·7	17·6	13·6
66	2700	12·8	10·9	8·1
30	2900	9·2	7·0	3·6

Disregarding temperature it is apparent that the most generally favourable relative humidities lie between 70 and 90 per cent. and the lower humidities (30 per cent.) are less favourable for the development of thrips larvae than a saturated atmosphere. The percentage of insects reaching maturity is low in all cases, but this, as previously recorded (4), cannot altogether be attributed to the unnatural conditions of the experiments, as even in the comparatively natural environment of a glasshouse,

under most favourable conditions of temperature and humidity (mean temperature 19° C., mean relative humidity 83·5 per cent.) the mortality of the insects is high. In an earlier paper in this series, Wardle (6) has shown that counts of thrips on American upland cotton, the type used in the present experiments, gave a ratio of adult insects to larvae of 9–16 in the glasshouse. In the incubator experiments the highest rate of mortality was among the late larval stages, but at almost every temperature and humidity a comparatively high percentage of prepupae became mature. As dead larvae are seldom found on the plants, it seems probable that, in a natural state the larva, after it has left the plant, and the pupal stage, which is also not found on the plant, are the most susceptible. In the incubator experiments almost all the prepupae reached a more adult stage, and as under favourable conditions the prepupal stage is very short, it is probable that the mortality rate in this stage is also low in the field.

In the experiments described in this paper, pieces of cotton leaf, free from any of the thrips larvae, were placed in the small tubes and 100 second-stage thrips larvae placed on them. The mouth of the tube was then closed with bolting silk and the whole tube placed in one of the larger tubes containing the salt solution. The large tube was then placed in one of the divisions of the multiple temperature incubator at a constant temperature. The temperature used ranged from 5 to 45° C., but owing to the limited space in the incubator it was not possible to find the percentage of larvae becoming adult at every degree of temperature for each of the five different degrees of humidity. It was endeavoured not to allow more than two degrees of temperature to separate experiments at the same degree of relative humidity. No adult insects were obtained at temperatures over 39° C., so temperatures of over 40° C. are ignored in the following tables.

The table on page 578 gives the percentage of larvae reaching maturity at each degree of temperature and humidity.

This table is given graphically in Fig. 1, and shows the percentage of larvae reaching maturity at constant relative humidity and varying temperature. From the graphs it is seen that the optimum temperature for the development of the larvae tends to rise with an increase in the atmospheric humidity, and that at high temperatures a correspondingly high degree of atmospheric humidity is necessary for the survival of the insect. At 85 per cent. relative humidity there is a large percentage of larvae becoming adult at 14° C., in fact, the number of mature insects (26 per cent.) obtained at this temperature was larger than at any other

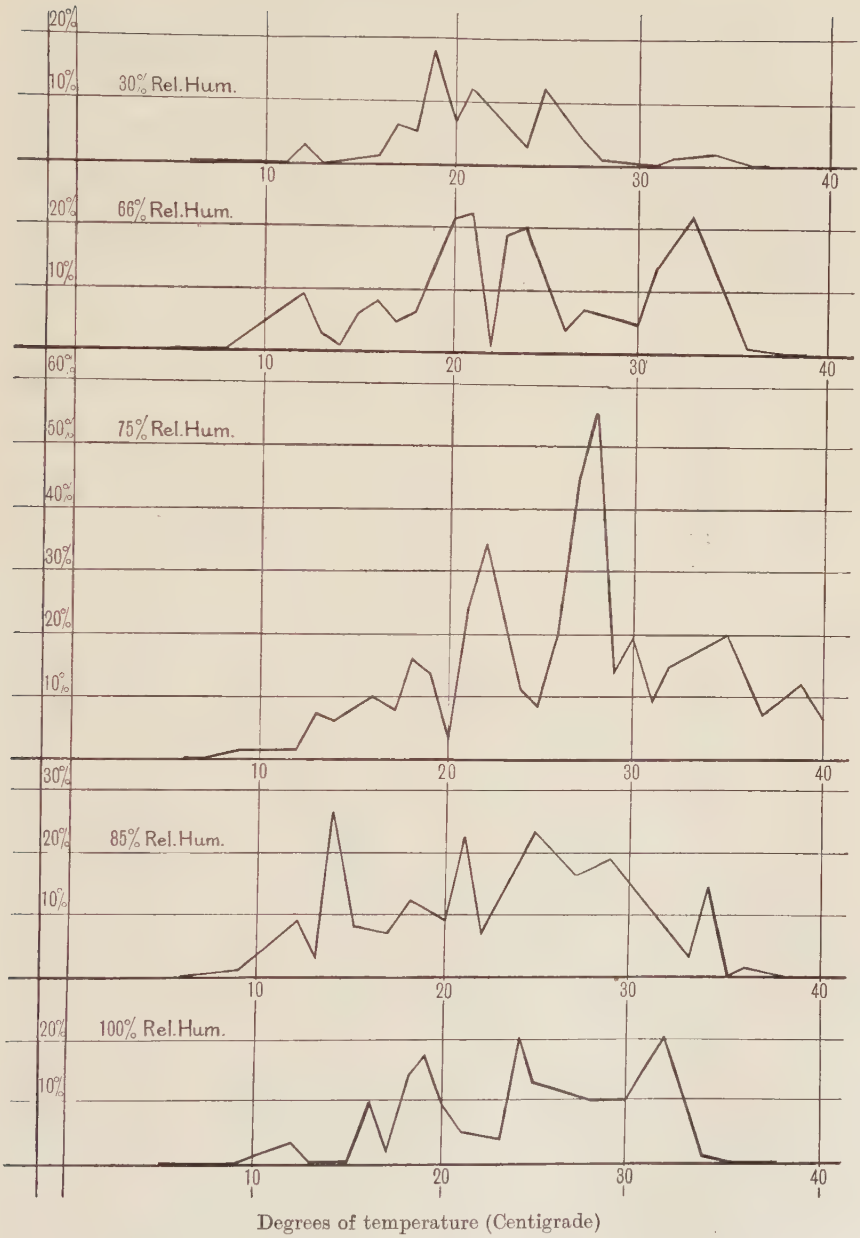

Fig. 1. Graphs showing the percentage of larvae reaching the adult stage at constant relative humidity and varying temperature.

for this humidity, but it will be seen from the table that this is an isolated instance and probably represents an abnormal batch of larvae, as the number of imagines which emerged at 14° C. was greatly in excess of any number obtained at temperatures immediately above or below. The highest temperature at which adult insects were obtained at any humidity was 39° C., and 9° C. was the lowest for any humidity; larvae reached the prepupal stage at 39° C. and as low as 5° C.; at 28° C. and 75 per cent. relative humidity 55 per cent. of the larvae became mature, a much larger number than at any other humidity.

	Relative humidity (%)				
Temp. (° C.)	100	85	75	66	30
39	—	0	12	0	—
38	0	0	—	0	—
37	—	—	7	—	0
36	0	1	—	1	—
35	—	0	19	—	—
34	1	14	—	—	2
33	—	3	—	22	—
32	20	—	16	—	1
31	—	—	9	14	0
30	10	—	19	5	—
29	—	19	14	—	—
28	10	16	55	—	1
27	—	—	45	7	4
26	—	20	20	4	—
25	13	23	9	—	12
24	20	—	11	20	3
23	—	—	—	13	—
22	—	7	34	1	—
21	5	22	24	22	12
20	9	9	3	21	4
19	16	—	14	—	18
18	13	12	16	6	5
17	2	7	8	5	7
16	9	—	10	8	1
15	0	8	8	6	—
14	0	26	7	1	—
13	0	3	7	3	0
12	3	9	1	9	3
11	—	—	1	—	0
10	—	—	—	—	—
9	0	1	1	—	0
8	—	—	—	0	—
7	—	—	0	—	0
6	0	0	0	—	0
5	0	—	—	0	—

In the table on p. 580 the range of temperature is divided into sections of five degrees and the percentage of larvae and prepupae reaching maturity is given.

Fig. 2 gives the table on p. 580 in diagrammatic form, and from these it can be seen that temperatures of 21–25° C. and 16–20° C. are the most generally favourable; temperatures of over 36° C. are definitely un-

favourable for the development of thrips larvae, though at this temperature more insects survived at 75 per cent. relative humidity than at any other humidity. In the temperature group 11–15° C. the proportions of the diagram are slightly distorted by the abnormal insects which have already been mentioned at 14° C. and 85 per cent. relative humidity, in the same way the percentage of adults obtained in the 31–35° C.

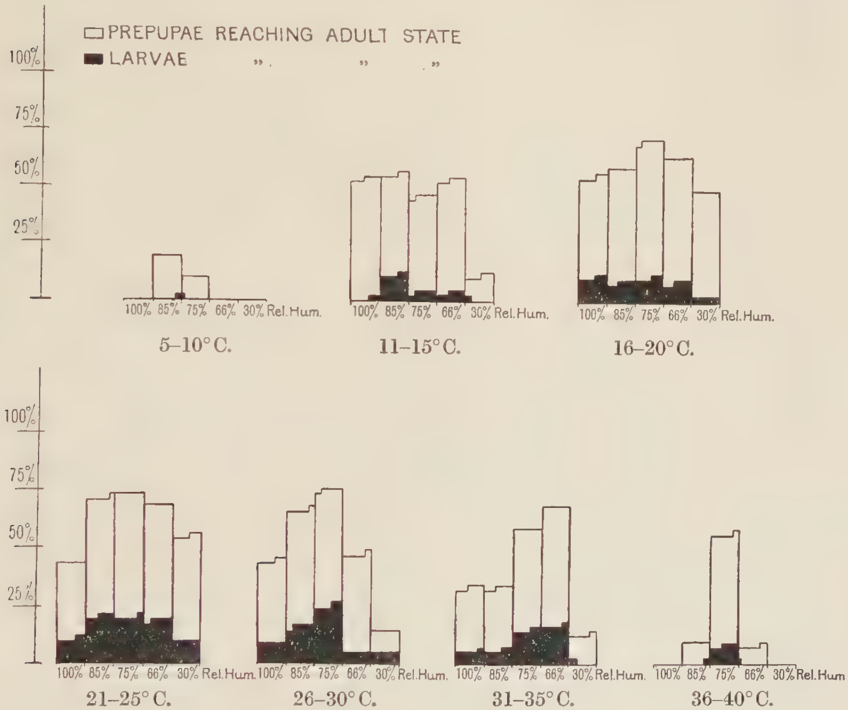

Fig. 2. Diagrams showing the percentage of larvae and prepupae reaching the adult stage at constant temperatures and varying relative humidity.

group at 66 per cent. relative humidity is probably rather too large. The diagrams emphasise the fact that 85 and 75 per cent. relative humidity give optimum conditions at almost all temperatures, with 66 per cent. relative humidity nearly as favourable. At relative humidities of 100 and 30 per cent. conditions are much less suitable for the survival of the larvae, though the insects are more able to withstand the too humid conditions of a saturated atmosphere than the dry atmosphere of 30 per cent. relative humidity.

	Relative humidity (%)									
	100		85		75		66		30	
Temp. (° C.)	Larvae	Pre-pupae	Larvae	Pre-pupae	Larvae	Pre-pupae	Larvae	Pre-pupae	Larvae	Pre-pupae
36–40	0	0	0·3	10	9	58	0·3	8	0	0
31–35	5·5	34	6	34	16	60	18	70	1	13
26–30	10	46	17	68	26	77	5	48	5	15
21–25	11	45	19	73	18	75	17	70	10	56
16–20	11	66	9	60	11	72	9	65	2·5	50
11–15	1	54	11	56	4·6	47	4	54	0·5	11
Below 10	0	0	0·5	20	0·3	10	0	0	0	0

Influence of humidity on the life cycle.

In the accompanying table (p. 581) and graphs (Fig. 3) particulars are given of the length of time (in days) occupied by the prepupal and pupal

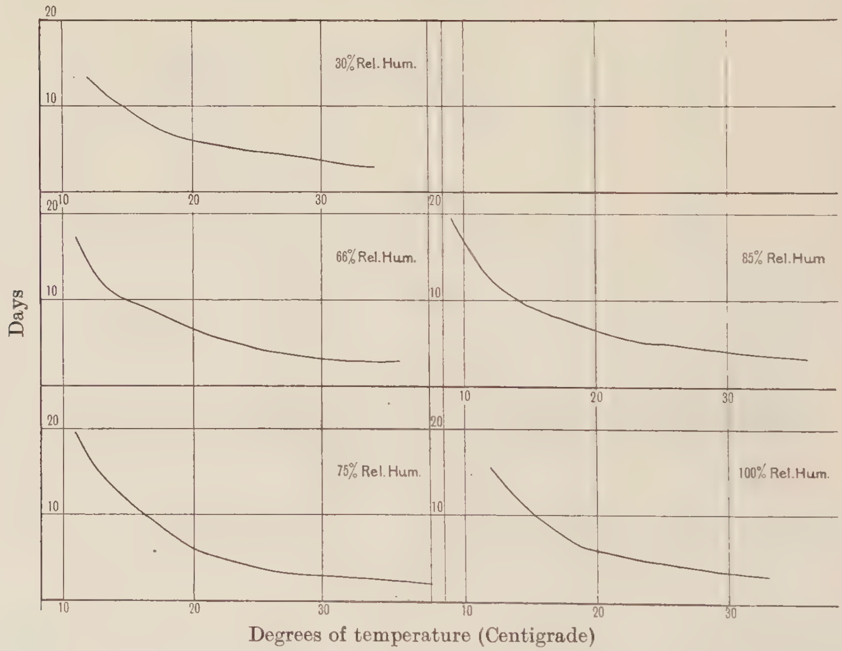

Fig. 3. Curves showing the length of the prepupal + the pupal stage at constant relative humidity and varying temperatures.

stages of *T. tabaci* at constant humidities and varying temperatures. It will be seen that while there is a considerable difference (10–15 days) between the length of these stages at low temperatures (9–11° C.) and high temperatures (34–39° C.) the curves for the different humidities

are all similar, which indicates that while the degree of relative humidity is of great importance in the question of the survival of the larva it does not seem to have any marked effect on the length of the life cycle of this insect.

Temp. (° C.)	Relative humidity (%) 100 (days)	85 (days)	75 (days)	66 (days)	30 (days)	Average length of prepupal + pupal stage
34	3·8	4·0	—	—	3	3·6
32	3·6	—	4·3	—	4·2	4·0
30	3·6	—	3·3	—	4·0	3·6
25	4·8	5·0	4·8	—	5·5	5·0
24	6·0	—	4·8	5·2	5·5	5·3
23	8·1	—	—	5·4	—	6·7
21	8·2	6·0	5·8	6·1	6·3	6·5
20	7·8	8·0	6·3	6·5	7·3	7·2
18	9·1	8·5	8·1	9·7	9·8	9·0
17	10·6	7·9	9·0	8·1	8·1	8·7
16	10·4	—	11·1	10·1	8·8	10·1
15	—	9·0	12·6	9·4	—	10·3
14	—	12·4	11·1	12·2	—	11·9
13	—	17·4	14·3	13·2	—	14·9
12	15·7	12·7	15·2	17·4	14·8	15·1

The largest variation in the length of these stages at any one temperature is 4·7 days at 12° C., and in most cases it is very much less than this; the largest variation at comparatively high temperatures was 2·7 days at 23° C.; in no case was it possible to trace any correlation between the variation in length of the prepupal and pupal stages and the degree of relative humidity.

INFLUENCE OF TEMPERATURE ON THE DURATION OF PREPUPAL AND PUPAL STAGES.

In the following table the range of temperature is again divided into sections of five degrees, and the lengths of the prepupal and pupal stages in each of these groups is given:

Temp. (° C.)	Length of prepupal stage (days)	Length of pupal stage (days)
36–40	Under 1–2	1–3
31–35	,, 1–3	1–5
26–30	1–5	1–6
21–25	1–6	1–9
16–20	1–7	2–10
11–15	1–9	4–14
Below 10	3–19	10–12 (9° C.)

The shortest time spent in the prepupal stage was less than 24 hours, and individuals with this short prepupal stage were found at temperatures of 35–40° C. At these same temperatures, pupae lasting only 1 day were common and 1-day pupae were found, though less often at

temperatures under 35° C., at all temperatures above 21° C. Prepupae of only 1 day were found at temperatures above 13° C., but between 13 and 21° C. these were not common, and many insects had prepupal stages of 4, 5 and 6 days. Two-day pupae were found at 17° C., but most of the insects had a longer pupal stage than this, 8 or 9 days as pupa was more usual at this temperature.

At the lower temperatures the length of the prepupal and pupal stages increased rapidly. The longest time spent in the prepupal stage was 19 days at 5° C., but another insect only remained in the prepupal stage for 7 days at the same temperature and neither insect became adult. In the present experiments the longest pupal stage was 15 days at 13° C., and the lowest temperature at which adults were obtained was 9° C. In another series of experiments, however, about seven pupae were found at 7° C., which took from 23 to 29 days before emerging as adult insects; in this latter series one prepupa, at 6° C., was 26 days before it reached the pupal stage, but this insect never became mature.

Conclusion.

From the results of the preceding experiments it is concluded that the degree of relative humidity has little or no effect on the length of the life cycle of *T. tabaci*, though it has a most marked effect on the mortality of the immature stages of this thrips. Superficially it may seem as though low humidities with high temperatures accelerate development, but in the case of *T. tabaci* this is due to the fact that those insects which do not transform quickly usually die before they can do so and thus the average length of the prepupal and pupal stages may be shortened, whereas, if the degree of relative humidity is higher (*i.e.* nearer the optimum) more of the insects survive and the average length of the prepupal and pupal stages is increased.

Delong and Mathewson (2), working on the development of an aphid, *Myzus houghtonensis* Troop, state that high humidity and low temperature retard development, while low humidity and high temperature accelerate it, but from the results obtained from the experiments described in the present paper it appears that this conclusion does not hold good for the Thysanoptera.

Summary.

1. It is found that in the development of *T. tabaci* the degree of atmospheric humidity is of great importance in the question of the survival of the larva.

2. At high temperatures a relatively high degree of atmospheric humidity (over 70 per cent.) is necessary if the insects are to reach maturity.

3. Although temperature has a most marked effect on the length of the life cycle of *T. tabaci*, the degree of relative humidity appears neutral, neither accelerating nor retarding the development of this insect.

I should like to acknowledge my indebtedness to the late Prof. J. S. Dunkerly for his advice during the work described above, and to thank Dr H. W. Miles for his most helpful criticism.

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THE PROBLEM OF "POTATO SICKNESS"

A REPORT UPON CERTAIN EXPERIMENTS

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(With Plates XLII and XLIII.)

The experiments described in the following paper were of a co-operative nature and were carried out in part at the Plant Pathological Laboratory of the Ministry of Agriculture at Harpenden and in part upon farms elsewhere. A number of investigators have been concerned in the work, and the authors should therefore be regarded as reporting on behalf of their colleagues.

In this connection, it should be recorded that the field investigations were planned in consultation with a Committee of Advisory Entomologists, viz., Mr T. H. Taylor, Leeds University, Dr H. W. Miles, Manchester University, Mr E. E. Edwards, Harper Adams Agricultural College[1], and Mr A. Roebuck, Midland Agricultural College, each of whom also assisted in carrying out the experiments in the area with which he was concerned. The work at Harpenden was dealt with by various members of the staff, the mycological aspect being under the care of Dr G. H. Pethybridge and Dr A. Smith.

Finally, gratitude must be expressed to Dr R. A. Fisher, of Rothamsted Experimental Station, for his assistance in regard to the statistical treatment of the results, to Mr J. C. Wallace, Director of the Kirton Agricultural Institute, for providing both facilities for field experiments and also for large supplies of "potato-sick" soil, and to Mr W. E. Gelling, District Lecturer in Agriculture at Selby, for his assistance with the field investigations there.

The nature of the problem.

In districts where potatoes have been grown continuously for a number of years, or where only a short rotation is practised, it has frequently been noted that sooner or later patches of sickly plants appear in the field. The plants in these patches are very much stunted, with

[1] Until 1928 Mr Edwards was Entomologist at the Kirton Agricultural Institute, and assisted with the experiments there.

weak, spindly stems; the foliage is pale in colour and slightly curly, and the lower leaves wither and drop off at an early stage. Underground, there is a marked reduction in the number of the fine fibrous lateral roots, while many of the smaller rootlets that arise directly from the stem are found to be dead. The tubers produced are few in number and very small, and the plants die down much earlier than they normally should.

In 1917 it was found by Taylor (1) that the roots of sickly plants in Yorkshire were heavily attacked by the eelworm *Heterodera schachtii* Schmidt, and this was confirmed in other areas in 1918 and 1919. In subsequent years the eelworm was invariably found to be present in sick plants, and it was generally considered to be the causative agent. A similar conclusion was reached by Continental workers, as exemplified in the writings of Zimmermann (2) and Reinmuth (3) in Germany, and of Kemner (4) in Sweden; a full bibliography will be found in the two last-mentioned papers.

In England, however, it has frequently been noted that normal-looking plants could be found, the roots of which were apparently as heavily infested by the eelworm as sickly plants in the same area, and in 1925 it was noted by Morgan (5) that fields showing great variation in the intensity of the disease did not have a corresponding variation in the number of cysts present in the soil. In the majority of cases Morgan's observations have been confirmed by later workers, although it was found by Smith and Prentice (6), in Lancashire and Cheshire, that where the disease was of recent appearance a positive correlation could be observed. Where the disease had been present for three or four years, however, no such correlation was found to exist.

In view of the observations recorded above, it was suggested by Morgan that some other factor, either connected with soil fertility or with fungus attack, might be a contributory cause of the sickness. The fungus most commonly present in the "sick" areas in Lincolnshire was *Corticium* (*Rhizoctonia*) *Solani*, and, as this fungus was also found in other districts where failure occurred, it was regarded as a possible factor in causing the "sickness." *C. Solani* has long been known as a parasite on the potato plant, but the damage attributed to it in this country has usually been confined to more or less serious attacks on the tips of the young sprouts emanating from the eyes, sometimes leading to entire "misses." Under certain circumstances it may attack the underground parts of the young stems and cause them to wilt and die. It may also, as first pointed out by Güssow (7), cause injury to the extremities of the

roots, and in dry climates or seasons it may cripple the plant in its later stages (rosette disease), but this is seldom the case in England.

In 1928, therefore, the position arrived at was as follows: appreciable areas of land in three important potato-growing districts, with smaller areas in other parts of the country, were proving no longer capable of growing a crop of potatoes, which crop, it may be stated, was in all cases the one greatly preferred by the farmers concerned. In spite of the importance of the failure thus disclosed, no satisfactory evidence had been brought forward to indicate the agencies actually responsible for the trouble; the eelworm theory appeared inadequate, the part played by the fungus *Corticium* was undecided, while, further, the possibility of there being some other causative agent had not been ruled out.

Consequently, it was decided that the problem must be approached afresh, with a view to establishing, if possible, the primary cause of the disease, and arrangements were made to re-investigate the position in the field and also to carry out more critical experiments in the laboratory. As the farmers in the areas concerned were in serious difficulties with the potato crop and were anxious that some immediate attempt should be made to discover a palliative, it was decided to extend the field investigations so as to include a test of the effect of dressings of naphthalene and any other cheap soil disinfectant which previous experience had shown might possibly prove useful. The present paper is intended to serve as a report upon the position reached by the end of 1930, chiefly in regard to the causation of "potato sickness." The trials of soil disinfectants are referred to in so far as they bear on this aspect of the matter, but it has been left to the investigator in each area concerned to deal with the more practical aspects of the trials.

Field investigations.

The field investigations consisted in the detailed examination of small, well-defined, typical "potato-sick" areas, and, where possible, adjacent "healthy" areas, in three of the districts chiefly concerned: viz. Lincolnshire, South Yorkshire, and the Ormskirk district of Lancashire. For the trials of chemical disinfectants (to be referred to later) each "potato-sick" area was divided into fifteen plots of $\frac{1}{20}$ acre each, while the "healthy" areas were divided into nine plots of the same size. It was possible, however, to secure healthy areas for experiment only in Lincolnshire and Lancashire. The previous crops in the different areas were as follows:

		1928	1927
Lincs. Kirton	Sick land	Potatoes then cabbage	Potatoes then cabbage
	Non-sick land	Sugar beet	Oats and barley
Yorks. Selby	Sick land	Swedes	Potatoes
Lancs. Irlam	Sick land	Potatoes	Clover
	Non-sick land	Clover	Oats

In the early spring of 1929, soil samples were obtained from the plots to determine the cyst population. These were taken to a depth of 9 in. with a soil auger, twenty-four borings being taken at random from each plot and, with the exception of those from the middle plots (No. 8) at each centre, mixed to form a bulk sample. The samples thus obtained were air dried, pulverised with a wooden roller, and sifted through a 2 mm. sieve. The percentage weight of the part that would not pass through the sieve was determined, the average percentages being as follows: Kirton nil; Selby 4·8 per cent.; Irlam 46·0 per cent.

Ten samples of 25 gm. each (10 gm. of the Irlam soil) were then drawn from each bulk sample, and the cyst content of each was determined. The method adopted was that of Morgan (*loc. cit.*) and consisted of shaking the sample in a conical flask filled with water, allowing the soil to settle, and collecting the cysts, which float to the surface, on a filter paper. They can then be counted easily under a low power binocular microscope. Owing to the large proportion of peat in the Irlam soil, and its consequent lightness, the samples from this locality presented some difficulty. It was finally found necessary to reduce the weight of the samples to 10 gm., and to float off the cysts in dilute alcohol.

Morgan's method of judging the potential eelworm population in the soil by counting the number of cysts present there during the winter is open to some objection, as there is a possibility that a varying number of the cysts are non-viable. This possibility was not lost sight of in the experiments described in the present paper, and a number of cysts were examined from each soil sample during the course of the counting. In every case examined living larvae could be obtained from the cysts, so that it may safely be assumed that the proportion of empty cysts counted was small.

The borings from each middle plot (No. 8) were sampled separately, as a check on the method, and to indicate whether the distribution of cysts varied greatly within so small an area as the plot. The variation so disclosed was not great, the samples from Selby showing a mean number of cysts of 122·5 per 25 gm., with a standard error[1] of $\pm$ 5·7; Kirton,

[1] Throughout this paper the standard error is calculated according to the usual formula, *i.e.*

$$\text{S.E.} = \frac{\sigma}{\sqrt{n}}, \text{ where } \sigma = \sqrt{\frac{s\,(x-\bar{x})^2}{n-1}}.$$

20·7 with a standard error of ± 1·2; and Irlam 28·3 (per 10 gm.) with an error of ± 1·0.

The plots were again sampled after the crop had been lifted, but the number of borings taken was then reduced to ten per plot, and the number of samples counted was only five, as the earlier results had indicated that the reduction in the standard error to be gained by taking twenty-four borings was neither essential to the experiment, nor commensurate with the additional work involved.

The following diagram shows the arrangement and numbering of the "sick" plots at Selby and Irlam; at Kirton the plan was substantially the same, but as the trials formed part of a larger experiment, for which the Kirton Institute was responsible, the arrangement differed slightly there.

Plot 13 Control	Plot 14 Creosote salts at 5 cwt.*	Plot 15 Naphthalene at 10 cwt.
Plot 12 Creosote salts at 10 cwt.	Plot 11 Creosote salts at 10 cwt.	Plot 10 Bleaching powder at 8 cwt.
Plot 7 Creosote salts at 5 cwt.	Plot 8 Control	Plot 9 Creosote salts at 5 cwt.
Plot 6 Bleaching powder at 8 cwt.	Plot 5 Napthalene at 10 cwt.	Plot 4 Creosote salts at 10 cwt.
Plot 1 Naphthalene at 10 cwt.	Plot 2 Bleaching powder at 8 cwt.	Plot 3 Control

At Selby it was not possible to secure a "healthy area" for the experiment, but at Irlam and Kirton such areas were obtained and were divided as follows:

Plot 22 Creosote salts at 10 cwt.*	Plot 23 Control	Plot 24 Bleaching powder at 8 cwt.
Plot 21 Bleaching powder at 8 cwt.	Plot 20 Creosote salts at 10 cwt.	Plot 19 Control
Plot 16 Control	Plot 17 Bleaching powder at 8 cwt.	Plot 18 Creosote salts at 10 cwt.

* The rates given for the various dressings are per acre in each case.

The nature of the different dressings will be seen from the two diagrams. Drained creosote salts, an impure form of naphthalene, was selected, as it had already been found by Edwards (8) to give promising results at Kirton. Naphthalene, Grade 16, was tested in order to discover whether the good effect was due to the naphthalene in the creosote salts or to the impurities present, while bleaching powder was considered worthy of trial as it was sufficiently cheap for the purpose and, moreover, was one of the few materials of this type which did not seem to have been tried sufficiently in the extensive work carried out on the Continent against *Heterodera* upon beet. The three chemicals were sown broadcast and the land was ploughed to a depth of 8 in. 14 days before planting. In all cases the standard manurial treatment for the district[1] was adopted, and the particular variety of potato popular in the district[2] and known to suffer greatly on potato-sick soils was planted, the dates of planting being April 12th at Kirton, May 28th at Irlam, and May 4th at Selby.

Table I. *Kirton.*

Plot no.	Treatment	Crop weight (cwt.)	Cysts per 25 gm. Before cropping	Cysts per 25 gm. After cropping
		Sick land		
1	Creosote salts at 5 cwt.	8·09	20·9±1·3	34·2±2·9
2	Creosote salts at 10 cwt.	9·56	28·1±2·5	26·0±1·4
3	Control	5·94	27·3±2·2	21·4±1·3
4	Naphthalene	8·38	16·5±1·3	23·6±2·6
5	Bleaching powder	6·60	33·7±2·8	24·6±2·5
6	Creosote salts at 10 cwt.	9·75	17·6±1·6	32·2±2·6
7	Creosote salts at 5 cwt.	8·41	22·6±1·3	31·2±1·9
8	Control	5·96	20·7±1·2	21·8±3·7
9	Naphthalene	7·14	17·5±1·3	22·4±1·7
10	Creosote salts at 10 cwt.	10·25	21·4±1·6	16·6±1·9
11	Bleaching powder	6·44	19·1±1·3	33·0±4·3
12	Creosote salts at 5 cwt.	7·50	23·1±2·5	21·8±2·2
13	Bleaching powder	7·84	15·7±1·3	14·6±1·6
14	Control	6·05	11·7±0·6	15·4±1·6
15	Naphthalene	9·03	23·1±2·5	27·8±2·2
		Healthy land		
16	Control	11·76	17·9±1·3	8·6±1·1
17	Creosote salts	11·29	26·1±1·3	19·0±2·6
18	Bleaching powder	9·37	26·8±2·2	7·8±2·3
19	Control	11·75	11·5±1·3	10·4±0·8
20	Bleaching powder	9·91	13·8±1·9	2·8±1·0
21	Creosote salts	11·20	23·2±2·2	7·0±1·4
22	Control	12·14	15·9±1·3	7·0±0·5
23	Bleaching powder	9·20	34·8±1·9	13·6±2·2
24	Creosote salts	10·39	20·4±1·3	5·4±0·6

[1] At Kirton, artificials consisting of 3 parts sulphate of ammonia, 4 parts superphosphate and 2 parts sulphate of potash, at 15 cwt. per acre; at Irlam, city refuse, indefinite quantity; and at Selby, farmyard manure at 15 tons per acre.

[2] Eclipse at Kirton and Selby, and Arran Chief at Irlam.

Table II. *Irlam.*

Plot no.	Treatment	Crop weight (cwt.)	Cysts per 10 gm. Before cropping	After cropping
		Sick land		
1	Naphthalene	7·16	36·8±2·2	72·2±3·6
2	Bleaching powder	5·79	34·6±2·2	62·6±5·7
3	Control	7·32	10·4±0·9	35·8±2·2
4	Creosote salts at 10 cwt.	7·88	11·0±1·6	29·8±3·9
5	Naphthalene	5·94	22·2±1·3	54·6±3·1
6	Bleaching powder	5·62	35·9±1·9	43·2±5·4
7	Creosote salts at 5 cwt.	9·81	31·4±2·8	44·6±3·2
8	Control	7·32	28·3±1·0	37·8±4·3
9	Creosote salts at 5 cwt.	7·59	10·4±1·3	28·8±3·1
10	Bleaching powder	6·88	12·3±0·6	37·8±1·9
11	Creosote salts at 10 cwt.	7·00	16·4±1·6	54·6±5·3
12	Creosote salts at 10 cwt.	6·30	42·0±2·2	57·4±5·3
13	Control	5·76	32·4±1·6	48·6±8·7
14	Creosote salts at 5 cwt.	5·73	34·5±2·2	57·8±8·7
15	Naphthalene	5·87	15·5±0·9	52·0±5·9
		Healthy land		
16	Control	16·18	0·9±0·2	0·6±0·3
17	Bleaching powder	16·04	3·3±0·6	0·4±0·3
18	Creosote salts	16·46	1·1±0·2	0·2±0·3
19	Creosote salts	15·89	1·8±0·5	2·2±1·0
20	Control	17·00	2·4±0·4	3·8±0·7
21	Bleaching powder	16·43	3·2±0·5	3·0±0·7
22	Creosote salts	16·06	15·7±0·8*	2·2±1·0
23	Bleaching powder	15·07	1·2±0·3	2·0±0·9
24	Control	16·77	1·7±0·3	3·6±1·6

* Evidently a localised "pocket," showing a very high cyst content, was struck during the first sampling; the second sampling probably shows the truer figure.

Table III. *Selby.*

Plot no.	Treatment	Crop weight (cwt.)	Cysts per 25 gm. Before cropping	After cropping
		Sick land		
1	Bleaching powder	5·41	97·8±3·5	79·4± 5·8
2	Naphthalene	6·39	82·5±4·4	60·8± 4·2
3	Control	4·08	82·9±3·8	77·2± 8·8
4	Creosote salts at 10 cwt.	3·96	90·3±3·5	85·6± 8·8
5	Bleaching powder	5·82	90·2±5·7	92·2±12·2
6	Naphthalene	6·18	88·0±7·3	93·4±10·6
7	Creosote salts at 5 cwt.	5·19	105·6±2·8	112·2± 7·2
8	Control	2·78	122·5±5·7	112·4± 6·5
9	Creosote salts at 5 cwt.	3·65	112·2±6·0	93·6± 4·9
10	Bleaching powder	2·53	94·6±7·6	86·2± 3·6
11	Creosote salts at 10 cwt.	4·76	83·7±3·2	96·4± 9·2
12	Creosote salts at 10 cwt.	6·57	79·9±4·4	83·6± 8·0
13	Control	5·72	120·6±6·7	111·8± 6·2
14	Creosote salts at 5 cwt.	4·11	76·8±3·5	79·2± 4·7
15	Naphthalene	4·22	86·2±4·7	82·4± 7·9

The various plots were examined periodically throughout the season of growth; typical "potato sickness" developed to a greater or less

extent in all the plots on "infected" land, while the plants in the plots on the two healthy areas developed normally except that bleaching powder appeared to be definitely injurious at Kirton, but not at Irlam. The crop from each plot was lifted and weighed after the tops had ripened off. The results of the three experiments are summarised in Tables I–III, which give the total crop for each plot and the cyst content of the soil both before and after cropping.

It is not proposed to discuss in any detail here the effects of the different dressings, but it is necessary to point out that none of them gave any significant gain in crop except in the potato-sick plots at Kirton, where the naphthalene-treated plots showed a definite increase. Reference to the cyst content of the soil of these Kirton plots (Nos. 1, 2, 4, 6, 7, 9, 11, 12, 15) before and after cropping shows that the naphthalene did not reduce the cyst content of the soil, and, in view of this, it is interesting to note that on the healthy land at Kirton, no increase of crop took place as a result of naphthalene dressings—*i.e.* the beneficial effect occurred only on the "sick" and not on the "healthy" plots.

Next, it is apparent that there is no relation between the cyst content of the soil and the presence of the trouble. The plots on the sick land at Kirton, before cropping, showed a range of 11·7 to 33·7 cysts per 25 gm. of soil, with a mean for the whole area of 21·22. The healthy land showed an almost identical range, namely 11·5 to 34·8, with a mean of 21·15. The figures for the Selby soil, which are comparable with those for Kirton, show a range of 76·8 to 112·5 cysts per 25 gm. of soil, with a mean for the whole area of 93·5. After cropping, the figures for Kirton were: for the "sick" land 14·6 to 34·2 with a mean of 24·4, and for the healthy land 5·4 to 19·0, with a mean of 9·1. At Selby, the range was 77·2 to 112·4, with a mean of 89·7. The weight of crop produced on the sick land was similar at both centres, and the figures suggest that the plants at Selby were able to support a larger eelworm population than those at Kirton. The great difference in the cyst population at the two centres may possibly be explained by the difference in manurial treatment, at Selby, farmyard manure was used, and this might stimulate root formation to a greater extent than the artificials which were used at Kirton. This point will, however, be discussed in more detail in a later section.

It is difficult to suggest a reason for the reduction in the numbers of cysts in the healthy plots at Kirton; in fact, the contrary was to be expected owing to the abundant food supply available in the vigorous root systems of the plants.

The Irlam cyst counts are not comparable with those from the other centres, as the specific gravity of the soil is so much lower. The sick plots here, before cropping, showed a range of 10·4 to 42·0 cysts per 10 gm. of soil with a mean of 24·9. After cropping the cyst content had increased considerably, a range of 28·8 to 72·2, with a mean of 47·8 being found.

Summarising the field investigations of 1929, it may be stated that no additional evidence connecting the eelworm with the trouble was discovered; on the other hand, they did not indicate the presence of any other agent not previously detected as likely to be concerned in the matter.

Pot experiments.

The object of the experiments was to test the effect on the growth of potatoes of *Heterodera*, of *Corticium*, and of a combined attack by the two organisms, and the general design of the work was as follows: Soil from Kirton, known to be "potato sick" was divided into five different bulks:

(1) Potato-sick soil untreated.

(2) Potato-sick soil steam sterilised.

(3) Potato-sick soil steam sterilised and reinfected with eelworm cysts to the same extent as the unsterilised soil.

(4) Potato-sick soil steam sterilised and reinfected with *Corticium*.

(5) Potato-sick soil steam sterilised and reinfected with eelworm and *Corticium*.

It was thus possible to examine the effects upon the potato of the two parasites suspected of causing the trouble and at the same time to discover the results obtained by a partial sterilisation of potato-sick soil. The pot-culture method was adopted, large (12 in.) pots being chosen in order to allow the plants room for reasonable growth. Ten pots were used for each bulk of soil Nos. 2 to 6 just mentioned, and sixteen for No. 1. The procedure adopted in carrying out the experiment was as follows:

A quantity of "sick" soil was obtained from Kirton; this was thoroughly mixed, and during mixing small samples were taken at intervals from which the cyst content was determined. Mixing was continued until the samples showed an approximately uniform distribution of cysts. The final sampling showed a mean content of $12·0 \pm 0·3$ cysts per 10 gm. of dry soil. The soil had been air dried, and had lost 25 per cent. of its weight in consequence. A pot contained approxi-

mately 26 lb. of re-moistened soil, hence about 10,000 cysts were required to reinfect each pot in series 3 and 5. These cysts were obtained by flotation in water and trapping in a 60-mesh sieve. As they were collected, they were cleansed as far as possible from any fragments of organic matter that had also been trapped in the sieve. It was found that approximately 1680 cysts weighed 0·05 gm., thus 0·31 gm. were required for each pot.

The supply of *C. Solani* required for series 4 and 5 was obtained by superficially disinfecting (in 1 in 1000 mercuric chloride) a sclerotium of the fungus removed from a potato tuber. This was then washed with sterile water and planted on malt agar in a Petri dish. Transfers were made to agar slopes, and from these a number of half-litre flasks, each containing about 100 c.c. of sterilised potato pulp, were inoculated. The flasks were incubated at 22° C., and when ready for use the contents were washed free of loose starch grains, mixed, and approximately equal portions were given to the various pots.

The soil for the pots in series 2–5 was partially sterilised by steam at 100° C., steaming being continued for 1 hour after this temperature had been reached. After cooling the soil to air temperature, the cysts were mixed with it in series 3 and 5, and the fungus culture in series 4 and 5. All the pots were plunged in groups in a bed of ashes on a terrace with a south-westerly aspect (slightly screened by a low brick balcony), and eight pots of the unsterilised soil were similarly plunged at each end of the bed.

A single tuber of the variety Eclipse was planted in each pot on April 15th. The tubers selected were of approximately equal size; they came from an Irish source, and were believed to be free from virus diseases.

By May 29th considerable differences could be seen between the plants in the unsterilised soil (series 1) and the others. The former showed the typical stunted appearance of "sick" plants, with deformed foliage and weak, spindly stems. In the other four series, however, all the plants appeared normal, no difference being observed between those in pots inoculated with eelworm or *Corticium* and those in which these parasites were absent. The fungus, however, in its sporulating condition, could be seen well developed on the haulms of the plants in series 4 and 5, just above soil level.

The haulms of the plants in the unsterilised "sick" soil died down between July 6th and 20th; the remainder persisted until August 5th to 17th.

The plants were lifted after all had completely died down, and the crop from each pot was weighed separately. It was found that the roots of the plants in series 1, 3 and 5, were heavily infested with cysts: the majority of these were fully developed and were easily shaken off the roots. After the crop had been removed, the soil in each pot was thoroughly mixed and samples were again taken, to determine the change, if any, in the cyst content. The figures are given in detail in Table IV, and it will be noted that the crop from the plants in unsterilised soil averaged only 45 gm. per plant as against 400–500 gm. per plant in the four other series. The cyst counts show that while the population in the unsterilised pots had doubled, that in the sterilised series (No. 3), to which cysts were added, had increased five times, while where both cysts and *Corticium* were added (series 5) the cyst population increased three times. The greater increase in series 3 over series 1 is not unexpected, as the root development in the plants was so much better, and they were thus able to support a larger number of eelworms. It is, however, more difficult to explain the difference between series 3 and 5. Here there was no apparent difference in the growth of the plants, and the crops were similar; an equal increase in cysts was to have been expected in each case.

In 1930, potatoes were again planted in the pots, no manure or any other treatment being given. The same variety (Eclipse) was used and the planting date, April 15th, was the same as in the previous year. The results, however, were entirely different. The plants in series 2 (sterilised soil) and series 4 (sterilised soil and *Corticium*) again grew normally, though, doubtless owing to lack of manure, they were not so large as in the previous year. Those in series 1 (unsterilised soil) again failed, while those in sterilised soil to which cysts had been added in 1929 (series 3 and 5) hardly made any growth at all.

The comparative appearance of the plants in early June in each year is shown on Plates XLII and XLIII.

The crops obtained from series 1, 3 and 5, were negligible, only a few grammes of minute tubers per plant; while those in series 2 and 4 yielded approximately 200 gm. per plant.

Cyst counts were again made after the crop had been removed. The cyst population in series 1 was found to have changed little; in series 3 there was a slight decrease, while in series 5 there was a considerable increase, the figures being much closer to those in series 3 than they were in the previous year. The detailed figures of crops and cyst counts for the two years are given in the following table:

Table IV. *Result of Pot Experiments.*

Series	Pot no.	1929 Crop weight (gm.)	1929 Cysts per 10 gm.		1930 Crop weight (gm.)	1930 Cysts per 10 gm.	
1 Unsterilised soil	1	25	30·0± 4·0		14	29·0±4·3	
	2	30	20·0± 1·8		10	20·6±1·1	
	3	10	15·8± 0·9		15	19·6±3·9	
	4	55	24·6± 4·0		61	46·4±3·7	
	5	40	21·6± 2·2		44	50·2±5·7	
	6	35	21·4± 2·2		11	20·4±1·8	
	7	45	37·8± 3·8		13	39·8±7·4	
	8	45	25·4± 3·6		Pot removed		
	9	50	18·8± 1·8		18	27·2±4·3	
	10	65	20·8± 2·7		10	22·8±1·8	
	11	25	19·4± 1·5		12	20·8±1·4	
	12	80	21·4± 2·2		15	19·6±2·0	
	13	30	25·2± 2·7		14	23·0±2·6	
	14	70	25·6± 1·8		20	26·6±1·7	
	15	Plant removed	22·4± 3·1		18	21·8±1·5	
	16	70	29·2± 2·2		8	27·6±2·4	
2 Sterilised soil	1	450	10·6± 0·9	Cysts killed by sterili-sation	158	8	Cysts killed by sterili-sation
	2	470	8·4± 0·6		156	7	
	3	480	8·2± 1·3		340	5	
	4	440	8·2± 1·3		161	10	
	5	420	7·6± 1·6		170	5	
	6	430	9·6± 0·8		231	9	
	7	460	10·8± 1·0		162	8	
	8	460	10·6± 3·1		157	6	
	9	470	10·6± 1·6		110	8	
	10	500	8·6± 1·3		Pot removed		
3 Sterilised soil reinfected with Eelworm	1	470	66·6± 0·9	Corrected figures*	Plant removed	62·2±2·0	Corrected figures*
	2	510	48·6± 6·7		10	55·2±2·2	
	3	500	47·2± 2·7		10	51·8±4·3	
	4	450	66·4± 9·8		3	62·0±4·7	
	5	360	58·4±17·5		4	50·2±2·9	
	6	440	81·4± 9·4		11	60·0±6·6	
	7	400	52·4±10·7		9	57·2±6·7	
	8	360	53·8± 2·7		5	41·2±5·0	
	9	360	82·4±12·2		4	76·2±9·2	
	10	370	61·8±10·7		5	65·2±4·0	
4 Sterilised soil reinfected with *Corticium*	1	510	8·6± 1·8	Cysts killed by sterili-sation	174	8	Cysts killed by sterili-sation
	2	440	9·6± 1·1		250	6	
	3	360	8·2± 1·6		112	7	
	4	400	9·4± 0·8		230	9	
	5	505	11·0± 1·1		188	10	
	6	440	8·6± 1·6		252	9	
	7	425	8·4± 0·9		165	6	
	8	420	11·4± 1·8		240	9	
	9	365	7·8± 1·6		112	5	
	10	475	10·4± 1·9		158	5	
5 Sterilised soil reinfected with both Eelworm and *Corticium*	1	360	39·4± 8·5	Corrected figures*	7	52·2±1·7	Corrected figures*
	2	385	38·2± 5·8		9	53·8±3·7	
	3	450	31·6± 7·6		8	55·4±4·0	
	4	450	47·4± 4·9		12	46·0±1·5	
	5	365	41·0± 5·8		10	50·0±3·7	
	6	420	44·4± 7·1		2	50·6±5·3	
	7	425	26·8± 6·2		13	44·6±2·5	
	8	515	34·8± 5·8		10	42·2±4·1	
	9	425	37·2± 6·7		11	51·0±4·1	
	10	410	30·0± 4·0		6	54·2±3·3	

* The figures in series 3 and 5 are corrected by deducting the number of dead cysts (killed by the original sterilisation) from the total count. These numbers were obtained by taking the mean of the counts in series 2 and 4.

The results obtained in 1929, when heavily infested plants grew normally while those less heavily infested failed, suggested four possibilities:

1. That the eelworm has no part in causing the disease, its presence in "sick" areas being purely fortuitous.

2. That the primary attack on the plant is made by free larval eelworms which had hatched from the cysts prior to sterilisation; these, of course, would not have been replaced.

3. That the emergence of the eelworms from the cysts that were added to the sterilised soil was delayed owing to their removal from the soil for a period.

4. That a second factor, which is destroyed by partial sterilisation, causes the disease in conjunction with eelworm attack.

The second and third of these hypotheses were tested by carrying out an experiment in the winter of 1929. "Sick" soil was washed through a sieve which would retain all cysts but would allow larval eelworms to pass through. The soil passing through the sieve and the washings were mixed with sand, and potatoes were grown in the mixture in a greenhouse. After 21 days no eelworms could be observed in the roots, although potatoes grown at the same time in "sick" soil and in sterilised soil to which cysts were added both showed a heavy infestation, practically from the beginning of root development.

It was thought that some light could be thrown on the first and last possibilities if the eelworm cysts could be removed from the soil mechanically, instead of killing them by sterilisation, and it was found that this could be accomplished by means of a fine sieve. A further quantity of "sick" soil was therefore obtained from Kirton; this was air dried, pulverised with a wooden roller, and sufficient was passed through a sieve of $\frac{1}{90}$ in. mesh to fill twenty 12-in. pots. Approximately 90 per cent. of the soil passed through the sieve, and 10 per cent. of silver sand was added to replace the larger particles. Half of the sifted soil was then sterilised in a similar manner to that adopted in the previous experiments. Thus, ten pots of sifted soil and ten pots of sifted and sterilised soil were prepared, and these were plunged in a plot in the garden together with a further eight pots of air-dried but unsifted sick soil. After watering the pots with rain water, an Eclipse tuber was planted in each pot on April 15th. The plants that developed in the unsifted sick soil showed all the symptoms of sickness. Those in the sifted soil, both sterilised and unsterilised, grew normally at first, but by mid-June those in the sterilised sifted soil appeared considerably

better than the others. The appearance of the plants at this stage is shown on Plate XLIII, fig. 5.

After the plants had died down, they were lifted, the crops weighed and soil samples taken to determine the cyst content. It was found that the sieving had removed practically all the cysts, there being no infection of the roots in a number of the pots and an entirely negligible amount in the remainder. In the pots of unsifted soil the cyst content had increased from 10·8 to 31·5 per 10 gm. of soil. The crops from the sifted and sterilised soil averaged about 650 gm. per pot, as against 300 gm. for the sifted sick, and 30 gm. for the unsterilised soil. Detailed figures are given in the following table:

Table V.

Air-dried soil (original cyst content 10·8 per 10 gm.)			Dried and sifted soil (original cyst content trace)			Dried, sifted and sterilised soil (original cyst content nil)		
Pot no.	Crop (gm.)	Cysts per 10 gm.	Pot no.	Crop (gm.)	Cysts per 10 gm.	Pot no.	Crop (gm.)	Cysts per 10 gm.
1	8	22	1	580	1	1	630	0
2	37	33	2	190	0	2	560	0
3	30	27	3	155	3	3	710	0
4	25	35	4	180	0	4	810	0
5	74	29	5	390	0	5	795	0
6	28	33	6	520	1	6	550	0
7	380	48	7	485	2	7	625	0
8	16	25	8	330	0	8	625	0
			9	260	4	9	810	0
			10	130	0	10	745	0

Discussion of results.

The experiments previously described leave no grounds for continuing to suspect the fungus *C. Solani* as a primary cause of "potato sickness," and no further reference to it need therefore be made here. The results in regard to the eelworm *H. schachtii* are, however, very much more difficult to interpret. The following generalisations seem, however, to be justified:

(1) Typical potato sickness does not occur in the absence of the eelworm. This is shown by all field surveys that have yet been made, and it is borne out by the pot experiments.

(2) Sickness does not necessarily occur, even when the eelworm is present on the roots in quantity. Again, this is shown both by the field surveys and also by the pot experiments.

(3) There is no correlation between the cyst content of the soil and the degree of intensity of the sickness. Thus, in the pot experiments, sickness of an advanced type occurred in unsterilised soil containing 30 cysts per 25 gm., whereas growth was normal in sterilised soil to which an equivalent number of cysts had been added. Equally, in the field, sickness occurred in soils with a cyst content of from 11·7 to 33·7 per 25 gm., while normal growth occurred in soils with a cyst content of from 11·5 to 34·8 per 25 gm.

An explanation of these somewhat anomalous conclusions may be sought along two lines.

I. In the first place, potato sickness may be caused solely by *Heterodera*, and its appearance or non-appearance may be due to some varying factor in the mode or the time of attack by the eelworm.

II. Alternatively, the eelworm may be regarded as one of two (or more) factors which jointly cause the sickness.

In regard to either of these hypotheses, it may first be pointed out that the symptoms and characteristics of potato-sick plants point clearly to a failure or an inadequacy of the root system early in the life of the plant. The root systems of potato plants that are affected never develop normally, and, in extreme cases, are insufficient to allow the plants to survive. According to the first hypothesis, therefore, the inhibition of root development would be regarded as due solely to the attacks of the eelworm, and the variation in the incidence of the sickness in infested soil might be caused either (*a*) by differences in the *percentage* of eelworms that emerge from the cysts and the consequent degree of infection of the plants, or (*b*) by differences in the *time* of emergence of the worms, an early and simultaneous emergence resulting in serious damage and late or delayed emergence allowing the plant to develop a sufficiently extensive root system in spite of the attack. Some attention has been given to these two possibilities, and while it is difficult to disprove either conclusively, neither appears to be acceptable for the following reasons:

(*a*) The root systems of healthy plants growing in soil infested by the eelworm always carry a large number of cysts; as the emergence of the females from the rootlets is not simultaneous, no method has yet been devised of comparing statistically the relative number of eelworms in "sick" and "normal" plants, but so far as eye observation goes, the latter appear to be as heavily infected as the former. In the pot experiments, the root infestations of the plants in the sterilised soil to which eelworms had been added were very heavy, although the plants (in the first year of the experiment) grew quite normally. It seems almost

impossible, therefore, to explain the healthy growth of plants in infested (although not obviously "sick") soil by any failure of the eelworms to emerge from the cysts.

(*b*) It would seem to be inherently improbable that, in a single field, the eelworms should emerge early and simultaneously in certain patches (*i.e.* those affected by potato sickness), but should extend their emergence over a longer period, or should emerge later, in other areas of the field which show no evidence of sickness. This is especially the case when it is remembered that the "sick" areas gradually increase in size until the whole field becomes affected.

Some experimental evidence that a delayed or extended emergence of the worms from the cysts is not of importance in practice is given by the greenhouse experiments referred to on page 596, which showed that the absence of sickness in plants grown in sterilised soil to which eelworms had been added was not due to any delay in emergence of the eelworms from the cysts.

From the evidence at present available, there seem, therefore, to be definite reasons for rejecting the hypothesis that eelworms are the sole causative agent of potato sickness, and attention must therefore be directed to the alternative hypothesis which postulates that the eelworm is one of two (or more) "factors" which are jointly responsible. In the first place, it is highly improbable that any unknown factor will prove to be a parasitic fungus or bacterial disease attacking the plant itself; potatoes growing in "sick" soil and showing typical symptoms were examined periodically throughout the season, and in spite of every endeavour to trace one, no parasitic organism (other than the eelworm) likely to be accountable for the trouble was discovered.

Since, therefore, any second factor would seem not to be in the nature of a plant parasite, and since, also, it cannot be a physiological condition innate in a certain proportion only of the plants, it can only be some factor related to the soil itself. Such a possibility is clearly a subject for a separate investigation by the soil scientist rather than the plant pathologist, and it is only possible here to draw attention to those aspects of the work so far carried out that appear to bear upon the matter.

In the first place, the sterilisation experiments show that the soil used responds very markedly to steam sterilisation, so that plants grown in it after such treatment can develop normally, even when suffering from an eelworm attack which in unsterilised soil is associated with their death. Secondly, this sterilisation effect is of a very temporary

character, the soil reverting to the "sick" condition in a single year[1]. Finally, the field trials with naphthalene again suggest that soil sterilisation phenomena are concerned. Naphthalene is known to be a partial soil-sterilising agent that is somewhat irregular in action (probably owing to its being broken down rapidly under certain conditions through bacterial action). At Selby and Irlam naphthalene had no appreciable effect upon potato-sick soils either as regards the crops produced or their cyst content. At Kirton, on the other hand, it produced a notable gain in crop, but it had no effect on the cyst content of the soil or on the apparent degree of infection of the plants; it would seem, therefore, that the only explanation of these observations is that the naphthalene, when effective, has acted as a partial soil-sterilising agent and not as a soil vermicide.

Such evidence as is available, therefore, suggests that the second factor (although the term "factor" is perhaps too concrete) is connected directly with the phenomena associated with partial soil sterilisation, and that it is concerned with the nutrition of the plant in its early stages. It is evidently not a mere lack of the essential mineral constituents of the soil, since complete artificials were employed at Kirton, and applications of lime had no effect in experiments carried out by Morgan (*loc. cit.*) in Lincs. and by Smith and Miles (10) in Lancs.; on the other hand, it is not impossible that one or other of these constituents, probably nitrogen, is not sufficiently available to the plant in its early stages of growth, and as a tentative hypothesis it is suggested that "potato sickness" is due to an infestation of *H. schachtii* in combination with some nutritional defect not at present apprehended.

Summary.

1. A study of the causes of "potato sickness" has been made during the past two years, both in the field and by means of pot experiments.

2. The sickness does not occur in the absence of the nematode *H. schachtii*, but potato plants may grow normally even when heavily attacked by this eelworm. No correlation was found between the cyst content of the soil and the intensity of the sickness.

3. There is no reason to suspect that the fungus *C. Solani* plays any primary part in causing the sickness, and no other parasitic organism (apart from the eelworm) likely to be responsible for the trouble was discovered.

[1] A similar result was obtained in pot experiments at Kirton by Cheal(9).

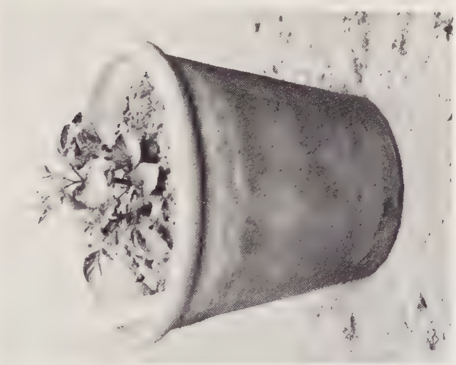

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

BUCKHURST AND FRYER.—The Problem of "Potato Sickness" (pp. 584–601).

Fig. 1.

Fig. 2.

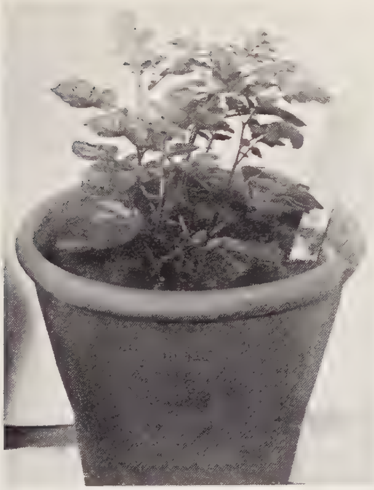

Fig. 3.

Fig. 4.

a *b* *c*

Fig. 5.

BUCKHURST AND FRYER.—THE PROBLEM OF "POTATO SICKNESS" (pp. 584–601).

4. The evidence suggests that the sickness is due to an attack by the eelworm in conjunction with a soil factor that inhibits a vigorous early growth of roots; it is possible that this second factor is a nutritional defect which is not made good by the ordinary manurial measures adopted in potato-growing districts.

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EXPLANATION OF PLATES XLII—XLIII.

PLATE XLII.

Fig. 1. Potato in untreated "sick" soil, 1929.

Fig. 2. Potato in sterilised "sick" soil, 1929.

Fig. 3. Potato in "sick" soil sterilised and reinfected with *Heterodera schachtii*, 1929.

Fig. 4. Potato in same soil as in Fig. 1, growth in 1930.

Fig. 5. Potato in same soil as in Fig. 2, growth in 1930.

Fig. 6. Potato in same soil as in Fig. 3, growth in 1930.

PLATE XLIII.

Fig. 1. Potato in "sick" soil, sterilised and reinfected with *Corticium Solani*, 1929.

Fig. 2. Potato in "sick" soil, sterilised and reinfected with *Heterodera* and *Corticium*, 1929.

Fig. 3. Potato in same soil as in Fig. 1, growth in 1930.

Fig. 4. Potato in same soil as in Fig. 2, growth in 1930.

Fig. 5. Potatoes in (*a*) untreated "sick" soil; (*b*) "sick" soil sifted to remove eelworm cysts; (*c*) "sick" soil sifted and sterilised.

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PYRETHRUM FLOWERS

A QUANTITATIVE STUDY OF THEIR DEVELOPMENT

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(With Plate XLIV and 9 Text-figures.)

CONTENTS.

Introduction.

Until the researches of Staudinger and Ruzicka (7) had demonstrated the constitution of the active principles of pyrethrum (*Chrysanthemum cinerariaefolium*) there was no method except that of direct biological trial that could be regarded as satisfactory for evaluating the flowers; and although biological methods have in recent years been brought to

a moderately high degree of exactitude, they require for accuracy a knowledge of a rather complicated technique. In the past rule-of-thumb methods of evaluating, having little to justify them except tradition and rapidity, have been practised. Chemical methods of analysing pyrethrum which have been recently worked out, although, no doubt capable of further improvement, do represent an advance in the means of evaluation previously available and have given results commensurate with the toxicities obtained by biological methods.

The present investigation was undertaken to follow the development of the flowers of pyrethrum and to ascertain, if possible, the way in which the pyrethrin content varies with the degree of development of the flowers and from plant to plant grown from the same seed in the same soil. The two main objects are, in practice, to some extent conflicting in the sense that for genetical study the larger the variations the more suitable in general the material for selection, whereas, the greater the heterogeneity the less valid the conclusions arrived at with respect to the correlations between the amounts of the active principles and the degree of maturity of the plant. The amount of material required for the analyses has an important bearing on both these aspects, since if large quantities are required it is only possible in exceptional cases, where the yield of flowers is high, to ascertain the degree of variation between the flower heads of one plant, and also since it may, in the early stages of development, render necessary the employment of the product of more than one plant and thus make difficult or impossible a free statistical examination of the data.

It has been the practice in the past to describe and differentiate between samples of pyrethrum flowers in terms of the degree of openness of the flower head, open, half-open, and closed buds being the categories into which the samples were separated. There does not, however, seem to have been any systematic attempt to define these categories, and many people are still in doubt as to whether these ascriptions refer to flowers immediately after harvesting or after being subjected to drying. For many years a superior value was attached to certain of these categories, and the fully open flowers were regarded as inferior. It becomes, in the first instance, a matter of importance to have a precise definition of what is meant by these terms, as, in the trade, they seem to be based upon the external characteristics in dried and withered flowers. We have made some attempts by photographing heads immediately after harvesting and again when completely air dry to ascertain how the external appearances have changed in the process of drying. In the

work outlined here the categories of maturity refer only to fresh flowers, as it was noted that the external character of the dry and withered flower heads depended upon small factors which, as yet, we have not been able to specify accurately.

Experimental.

In the grounds of the Plant Pathology Laboratory of the Ministry of Agriculture, Harpenden[1], a bed running north and south, 27 by $9\frac{1}{2}$ ft. was planted with 108 pyrethrum seedlings on July 13th, 1928. The history of the bed as far as recorded is as follows:

Dec. 1926. Moderate dressing of farmyard manure.

April 1927. Potatoes planted.

Oct. 1927. Potatoes dug.

July 4th, 1928. Plot forked over and two barrow loads of coal ashes passing $\frac{1}{4}$ in. sieve and two bushels of slaked lime added.

$25\frac{1}{2}$ ft.

S $9\frac{1}{2}$ ft. N

1				2				3				4			
.*E*	.*B*	.*D*	.*C*	.*A*	.*F*	.*D*	.*G*	.*B*	.*G*	.*F*	.*H*	.*C*	.*A*	.*H*	.*E*
.*F*	.*G*	.*A*	.*H*	.*B*	.*H*	.*C*	.*E*	.*D*	.*E*	.*C*	.*A*	.*D*	.*F*	.*B*	.*G*
5				6				7				8			
.*F*	.*A*	.*G*	.*C*	.*C*	.*A*	.*G*	.*D*	.*F*	.*G*	.*E*	.*A*	.*F*	.*C*	.*E*	.*B*
.*H*	.*D*	.*B*	.*E*	.*F*	.*H*	.*B*	.*E*	.*H*	.*D*	.*B*	.*C*	.*A*	.*G*	.*D*	.*H*
9				10				11				12			
.*B*	.*G*	.*H*	.*D*	.*B*	.*D*	.*G*	.*A*	.*D*	.*G*	.*H*	.*C*	.*G*	.*B*	.*D*	.*A*
.*C*	.*F*	.*E*	.*A*	.*C*	.*H*	.*E*	.*F*	.*A*	.*E*	.*F*	.*B*	.*C*	.*E*	.*H*	.*F*

Plan of bed.

The plants weathered the hard winter of 1928–9 and in the spring of 1929 appeared in most cases to be well established. The two rows of plants at the south end of the bed were marked off from the remainder (leaving $25\frac{1}{2}$ ft. of bed), which was then divided up into twelve blocks of eight plants each. The plants in each block were selected at random and marked with the letters *A*–*H*. The plan of the experiment was to take the flower heads from one plant per block each week, beginning with *A* in the small bud or button stage, and proceeding in this fashion over a period of 8 weeks until finally the *H* plants were taken in the overblown

[1] I am indebted to Mr J. C. F. Fryer, for permission to use this bed and to him and Mr C. T. Gimingham for their constant care and attention to the plants, and for advice during the course of the work.

state. In reality, a rather longer period than 7 days (10 days 19 hours) had to be allowed before the flowers on plants *H* could be regarded as completely overblown. One of the *G* plants (in Block 2) died, and in addition the test on plant *C* in block 4 was spoilt, thus one of the plants had to be wholly and the other partially ignored. The configuration of the bed is given in the plan.

In the plan, the letters were given to assist randomisation and facilitate the proper selection of the plants each week; in the tables, however, in order not to confuse the procedure and to indicate more clearly the week in which the flowers were taken, the plants are given numbers corresponding to the number of the letter in the alphabet. Thus the crop from all the *A* plants taken on May 28th are called the first week's, the *B* plants the second week's and so on down to *G* the seventh week's, while that of the *H* plants, left 1 week 4 days after the collection of the *G* plants, roughly a week and a half, is specified as taken after $8\frac{1}{2}$ weeks.

By the middle of May it was observed that flower buds were forming on the plants; they were, however, too small to give a sufficient weight for analysis. On May 28th the first crop was taken, a certain length of stalk (12–18 in.) being cut, the product of each plant being kept separate, the heads with the stalk were air-dried in the shade in a greenhouse, the temperature of which was not allowed to rise too high and through which a circulation of air was allowed to pass by opening doors and windows. For the first three weeks the heads were all buttons, but on the fourth petals began to emerge. Up to and including the fifth week the heads were separated, after drying, into categories to correspond with their condition at the time of harvesting. This was a comparatively easy matter up to this stage, but afterwards the material was separated immediately after harvesting and the heads in the categories dried separately. After drying, the heads were cut off and stored either in corked tubes or air-tight tins, and the diameter of the receptacle of each head subsequently measured; the receptacles were nearly all practically circular, but in cases where there was a lack of symmetry the mean diameter was determined from two measurements. A piece of cardboard with a V-shaped cut in it and having graduations down the side greatly facilitated measurement in the early stages, but when the flowers were fully open or overblown, callipers were employed. The gross weights of the heads in the different categories were then determined. During the process of measuring it was convenient to detach the last remnant of the stalk, and thus the analyses represented results for flower heads alone. They may have on this account a higher pyrethrin value than

those usually found for large samples where it would be almost impracticable to detach the stalk so completely. The advantages, however, of this practice are clear, as there is no confusion likely to arise owing to one sample having a proportionally larger quantity of stalk than another.

The categories. The use of the terms buttons, closed with petals, half-open and overblown heads requires some definition. The difficulties involved in this have already been indicated. In Plate XLIV, however, we have given photographs illustrating the several classes from the button to the fully open stage. Plate XLIV, fig. 1 A, shows them as harvested. Reading from the left we have, first the closed bud or button stage; the two following flowers show the range covered by the term "closed showing petals," the flower 3 being in a more advanced stage than 2; the fourth flower we have regarded as "half open" but in rather an advanced stage, and this category would, in general, include a range of flowers down a stage where the ray florets had just flattened out. The last flower to the right would be regarded by us as just "fully open," the first ring of the disk florets having opened out, but as it was impossible to select flowers in precisely this state, the range would include flowers in which several rings of disk florets were open. Plate XLIV, fig. 1 B, illustrates these flowers after air-drying, and it is obviously not easy by casual inspection to differentiate between the later classes when dry. Plate XLIV, fig. 2, shows the fully open flowers just after harvesting (A) and when air-dried (B) respectively. In the air-dried series (B) it will be noted that the first three flowers to the left have dried with the petals closed over the disk, whereas the three to the right have dried outwards and downwards exposing the disk florets to view. Recording the flower on the extreme left as No. 0, in which no disk florets are open, and proceeding to the right, No. 1 has the first circles from the periphery open, No. 2 the first and second circles, No. 3 all open to the third circle, No. 4 is open to the fourth circle, and in No. 5 all the disk florets are open except a few in the centre. It would appear, therefore, that there is a tendency on drying for the petals to shrivel over and to enclose the head until a certain number of the circles of florets (about half) are open, and when maturity has passed beyond this stage for the petals to shrivel outwards and backwards. It is, however, almost certain that a considerable variation will be found in this regard, and without the examination of a large number of flowers, it would, in the present stage of our knowledge, be unwise to rely upon this characteristic as more than a rough indication of maturity. As a good deal of heterogeneity

exists even in a small bed of pyrethrum flowers, it must be assumed that without close inspection flowers ranging over all the above six classes would be taken as fully open.

Plate XLIV, fig. 3, represents flowers in the completely overblown stage; there is no great difference between the appearance of the flowers before and after air-drying.

Meteorological conditions. The weather conditions prevailing during the period of growth of the flowers was remarkably uniform. Little rain fell over many months, and the temperature both of the air and soil was very uniform. The highest temperatures occurred during the last 11 days of the experiment, the maximum averaged over that period being 76° F. and the minimum 52° F.; usually, however, the maximum daily temperature ranged about 62–67° F. and the minimum about 45–50° F. The soil temperatures were slightly lower. The hours of sunshine and the total radiation as Callendar figures, calculated to joules, are given in Table I.

Table I.

Sunshine and radiation figures.

Week	Bright sunshine (hours)	Radiation figures (joules)
1–2	44·8	10,576
2–3	55·8	11,664
3–4	50·1	11,314
4–5	63·4	12,391
5–6	40·8	10,792
6–7	41·1	9,441
7–8½	131·6	22,812

It is possible that the uniformly dry and moderately warm weather prevailing during some critical period of growth had the effect of raising the pyrethrin content of the flowers. The determination of the effect of meteorological conditions, particularly of rainfall, temperature and sunshine, upon flowering and the content of the pyrethrins in the flowers, would appear to be a matter of some importance. It is known that this plant rarely, if ever, flowers in certain countries close to the equator. A direct experiment to determine the meteorological conditions that lead to maximum and minimum flowering and content of the active principles might be of value as indicating the types of climate suitable or unsuitable for the growth of this plant.

The total radiation figures and the sunshine hours for each period are plotted in Text-fig. 1 and the accumulated data, counting from the first period, in Text-fig. 2. There is a considerable rise in the figures during

the last week and a half, and this is concordant with a large increase in the weight of the flower heads during this period. It is doubtful whether the correlation is important, as prior to and during this time pollination has taken place and the fruits are forming and a large increase was to be expected. It is, however, a moot point whether it would have been so rapid except for the large amount of sunshine experienced during the period. A correlation between the amount of radiation and the increase in weight and of the pyrethrin content during the periods was not sought, and it would appear as if only a direct experiment in which all the other variations likely to occur in the open are reduced to a minimum could elucidate the part played by sunlight in the development of the flowers and their active principles.

The estimation of the pyrethrin content. The samples were ground to a moderately fine powder and thoroughly mixed for analysis. An attempt was made to analyse the material from individual plants for the third week, but the spoiling of one test and the impossibility of repeating the analysis rendered the pooling of the material necessary. Four individual plants had been analysed in this case, but the remainder of the samples were mixed together, the pyrethrin content determined and the mean values for the pyrethrins calculated for the whole (eleven plants). In view, therefore, of the small amount of material available from individual plants for the first two weeks, flowers for each of these weeks were mixed together, ground and the pyrethrin content determined. In the subsequent weeks the samples from each plant were ground and analysed separately, and in two cases, to be dealt with later, it was possible to do estimations on the flowers in the separate categories. The loss on heating to 103° C. in an electric oven for 24 hours was ascertained, and although it is known that a small amount of volatile oil is present in the fresh flowers, the material after this treatment was regarded as dry matter and the pyrethrin contents per cent. were calculated to this basis. These values are stated in the tables as percentages on the oven-dried heads.

The samples were extracted with petroleum ether (B.P. under 40° C.), and the contents of pyrethrin I and II determined by the micro-method outlined by Tattersfield, Hobson and Gimingham (8). The values, however, came out in the later weeks at so high a level that difficulties were met with in the analyses and some slight modifications of the method had to be introduced. This phase of the problem has been dealt with in a separate paper by Martin and Tattersfield (4). In addition, the copper-reduction method outlined by Gnadinger and Corl (2) and the ferricyanide

method of Martin and Tattersfield (*loc. cit.*) were also employed for checking certain values that appeared unusually high. The latter method was devised as a result of the difficulties experienced in dealing with our samples which, in some cases, were much richer than we had hitherto met with; it enabled us to check rapidly the results obtained in the later stages of the growth of the flower heads, but was not so successful for analysing the small buds. The pyrethrin content is expressed in the tables as percentages on both the air-dried and oven-dried heads, and also as the mean amount found per flower head and per plant. There were considerable variations in the values obtained for the flower heads from individual plants in all the weeks where it was found possible to carry out analyses.

In order to test the significance of the results obtained a statistical analysis of the data was carried out by the Statistical Department at Rothamsted. This not only makes it possible to decide how far reliance can be given to the conclusions drawn, but also to put on record in an abbreviated form the large mass of figures accumulated, while giving some indication of the degree of variation existing amongst the detailed data. An analysis of variance, the main procedure employed (1), makes possible through the "z" test a determination of the significance of the effect of position of any plant in the bed, and of time of harvesting upon the value being considered and gives a relatively more accurate estimate of the standard errors of single plants or those of the means of twelve plants. Where, however, the data were not suitable for the application of this analysis the standard errors were determined in the usual way. It may be noted here that, when tested, the standard errors of the general mean were not significantly different in value from those determined for the means by plants.

Results.

Numbers of flower heads. The whole of the flower heads on each batch of plants were taken on each occasion. Over the whole of the series there was a considerable amount of variation, the lowest yield on one plant being 18 and the highest 440. The mean numbers per plant based on the crop taken each week are given in Table II.

The analysis was carried out to ascertain whether the mean numbers of flowers varied significantly with the blocks into which the bed was divided or with the dates upon which the flowers were gathered. The "z" test indicates that there was just a significant block effect, which

varied from 153 to 76 per cent. of the mean yield. There was no effect due to dates, *i.e.* the number of flower heads found are not likely to vary significantly whether taken the first week or the last. It is obvious that the flower buds are laid down in the earlier stages of the annual growth and do not materially increase in number during the course of the experiment.

Table II.

Numbers of flower heads each week.

Week	1	2	3	4	5	6	7	8½
Mean nos. per plant ...	136·2	163·2	155·7	157·7	145·9	162·7	138·7	159·6

Standard error of a single plant $=\pm 63{\cdot}28$; standard error of mean of twelve plants $=\pm 18{\cdot}27$.

An analysis of variance gave the following results:

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$ mean square
Blocks	11	87,397	7945	1·0362 ($z=0{\cdot}3427$)
Dates	7	9,476	1353·7	
Remainder	75	300,285	4003·8	0·6936
Total	93	397,158		

Degree of maturity of the flower heads. In Table III an attempt is made to give a short representation of the degree to which the maturity of the flowers varied with the date of harvesting. The general mean percentages for twelve plants for the flowers found in each category are given, and alongside them in brackets the range of percentage values among the individual plants. Thus, the flowers taken on June 25th from twelve plants gave a mean percentage of buttons of 8·3, but samples taken from each plant showed a variation ranging between 0·7 and 24·3 per cent. The system of tabulating was adopted since the value of a full table of figures was not commensurate with its complexity.

Table III.

Degree of maturity of the flower heads.

Week (Date of harvesting)	Buttons (mean %)	Closed showing petals (mean %)	Half open (mean %)	Fully open (mean %)	Over-blown (mean %)
1 (May 28)	100	—	—	—	—
2 (June 4)	100	—	—	—	—
3 (June 11)	100	—	—	—	—
4 (June 18)	83·6 (58·6–97·8)	16·4 (2·2–41·4)	—	—	—
5 (June 25)	8·3 (0·7–24·3)	64·7 (30·2–84·9)	20·1 (3·0–52·6)	6·9 (0–25·3)	—
6 (July 2)	2·1 (0–13·1)	16·6 (2·2–49·2)	12·4 (8·4–19·6)	68·9 (28·7–87·6)	—
7 (July 9)	—	2·1 (0–7·7)	6·4 (0–52·8)	91·5 (47·2–100)	—
8½ (July 20)	—	—	—	—	100

The figures represent the general means. The numbers in brackets give the minimum and maximum percentages found.

No attempt has been made to correlate the results expressed in Table III with the pyrethrin contents, but as the average degree of maturity is obviously correlated with the dates at which the flowers were taken, a significant effect on the pyrethrin contents correlated with the dates may well be one due to maturity. There is obviously a good deal of heterogeneity in the flowers taken in the fourth, fifth, sixth and seventh weeks, as judged by their degree of openness. The data for the flowers of the fourth week were tested statistically and a significant variation in maturity among its twelve plants was found. It is hoped that at an early date it will be possible to make a more accurate comparison between the rates of maturation of different plants and to ascertain to what extent genetical factors may be involved.

Size of flowers. It was considered that the diameter of the receptacles of the flowers would give a rough estimate of the size of the flower heads. These data had been accumulated in the way described for each head. The mean diameter of the heads from each plant, the general mean and the mean by plants for each series of twelve plants were calculated. The two latter means are expressed along with their standard errors in Table IV.

Table IV.

Mean diameter of receptacles of flower heads each week.

Week ...	1	2	3	4	5	6	7	8½
General means in mm. ...	4·07	5·59	6·20	7·17	7·86	8·71	9·4	11·7
Means by plants in mm. ...	4·03	5·66	6·21	7·31	7·89	8·81	9·31	11·8
Standard errors	±0·134	±0·135	±0·078	±0·184	±0·146	±0·176	±0·289	±0·264

An analysis of variance for this table was not carried out, but the standard errors for each week's crop were ascertained. The general means were determined by dividing the sum of the diameters by the total number of flowers gathered each week, while the means by plants were ascertained from the weekly sum of the means for each plant, divided by the number of plants from which that week's crop had been taken. There is little difference between these two values and no significant differences between their respective standard errors.

Moisture content of the flower heads. These were determined by drying the powdered flowers overnight in an electric oven heated to 103° C. For the first three weeks the crops from the whole twelve plants were mixed and used for the estimations, but, during the following five and a half weeks the crop of flowers from each plant was tested separately. The mean values are given in Table V.

Table V.

Moisture content of flower heads in percentages.

Week	1	2	3	4	5	6	7	$8\frac{1}{2}$
General mean values	11·6	11·2	10·3	10·3	10·7	11·4	11·8	10·6
Mean values by plants	—	—	—	10·65	10·61	11·38	11·78	10·52

Analysis of variance.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"
Dates	4	15·2326	3·8082	1·81982	0·82698
Blocks	11	7·6498	0·6954	—	—
Remainder	43	31·3194	0·7284	0·99284	—
Total	58	54·2018			

The standard error of a single plant as calculated from the remainder $= \pm 0·85$ per cent.
The standard error of the mean of twelve plants $= \pm 0·38$ per cent.

The dates upon which the flowers have been taken have had a significant effect, but no effect due to blocks is indicated.

The determination of the moisture content in this way probably gives only an approximate estimation, as the flowers contain small amounts of volatile oil, part of which would be lost at 103° C.; the loss due to this factor is, however, not large.

Weights of flower heads. The average figures for the weights of the heads are set out in Table VI.

Table VI.

Weights of flower heads at each harvesting.

Week of harvesting	Mean weight of air-dried heads per plant (gm.)	Mean weight of oven-dried heads per plant (gm.)	General mean weight of single air-dried head (gm.)	Mean weight by plants of single air-dried head (gm.)	General mean weight of single oven-dried head (gm.)	Mean weig by plant of single oven-drie head (gm
1 (May 28)	1·1568 ± 0·18	1·0226 (± 0·16)	0·0085	0·0084 ± 0·00066	0·0075	(± 0·0(
2 (June 4)	3·0396 ± 0·28	2·699 (± 0·25)	0·0186	0·0195 ± 0·00124	0·0165	(± 0·0(
3 (June 11)	4·759 ± 0·45	4·379 (± 0·4)	0·0313	0·0318 ± 0·00204	0·0256	(± 0·0(
4 (June 18)	9·075 ± 0·88	8·140 ± 0·78	0·0575	0·0594 ± 0·0027	0·0516	0·0531 ± 0·0(
5 (June 25)	17·267 ± 2·68	15·416 ± 2·38	0·118	0·115 ± 0·0037	0·106	0·103 ± 0·0(
6 (July 2)	25·531 ± 4·40	22·612 ± 3·87	0·157	0·158 ± 0·0054	0·139	0·140 ± 0·0(
7 (July 9)	25·964 ± 3·99	22·890 ± 3·53	0·187	0·180 ± 0·0095	0·165	0·1586 ± 0·0(
$8\frac{1}{2}$ (July 20)	41·312 ± 3·80	36·930 ± 3·32	0·259	0·262 ± 0·0092	0·231	0·2345 ± 0·0(

Note. The general mean was obtained by dividing the total weight of flower heads from the twelve p by the total number, the mean, by plants by dividing the sum of the means for each plant by the numb plants. There was not a significant difference between the standard errors of these means.

In Table VI, the average weight of heads per plant obtained from twelve plants is expressed for both the air-dried and oven-dried (at 103° C.) material. We were not able to ascertain the standard errors for the latter for the first three weeks as the samples had been pooled for

purposes of analysis, but it will be observed from the table that although the errors, where determined for the oven-dried heads, are slightly smaller than for the air-dried, there is a close proportionality, from which the values in brackets have been calculated. The remainder of the table gives the average weight of a single head from each week's harvest both for the air- and oven-dried material. The general mean and the mean by plants were obtained in the usual way. In the text-figures we have plotted only the general means of the oven-dried flowers. An inspection of Table VI shows that while, in general, there are rises in the yield of the flowers by weight per plant with the passage of time, the increase in weight between the sixth and seventh week is definitely not significant. It is at this stage that the flowers approach maturity and any considerable increase would not be expected, but the standard errors throughout this portion of the table are rather high, due to considerable variation as regards yield in numbers of flowers per plant and the weights of flowers. An analysis of variance showed that the yield of flowers by weight per plant was just significantly affected by position in the bed, but was profoundly affected by the date. The latter statement conveys the obvious truth that the flower heads will unquestionably increase in weight as they grow to maturity.

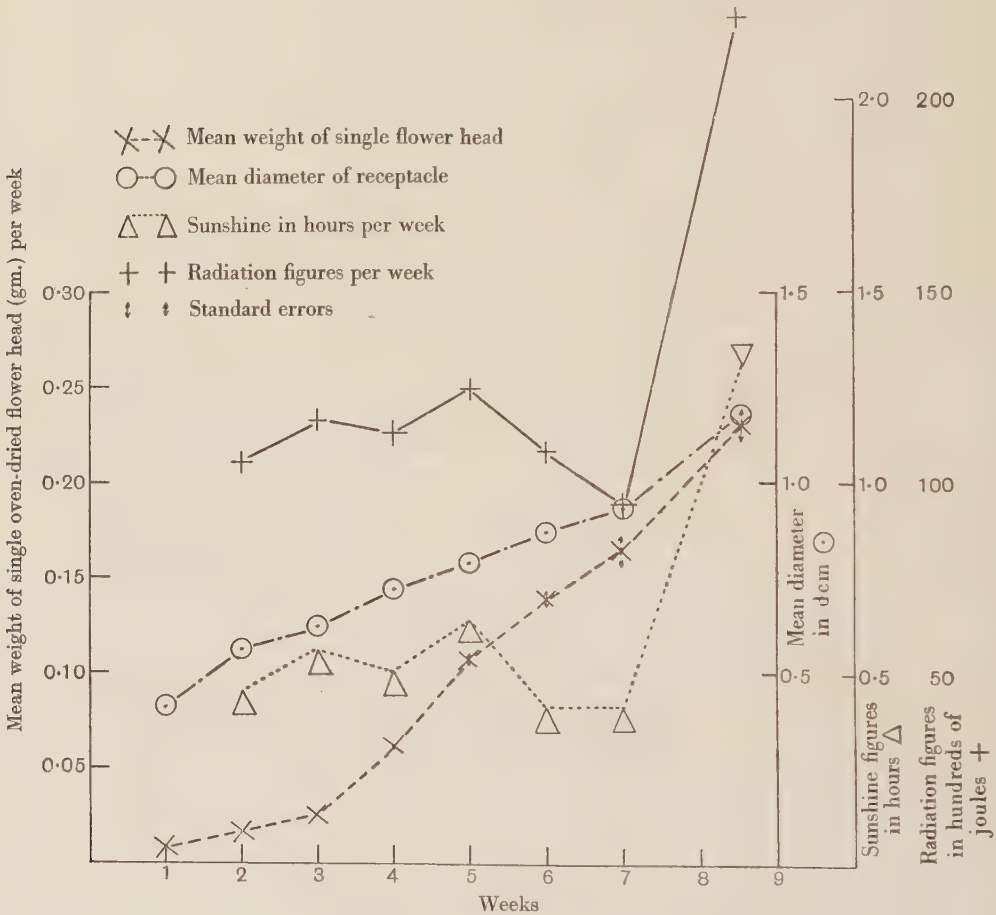
The data in Table VI show that the average weight of a single flower head each week at first increases slowly then more rapidly, and although retardation takes place as maturity of the flowers is approached there is rather a sudden rise after the seventh week, when pollination has been effected. The growth curve is S-shaped, up to the point where pollination has taken place. The weekly average weights of the single flower head are plotted in Text-figs. 1 and 2, together with the radiation figures.

No attempt has been made to seek a correlation between the radiation and growth data. On one season's figures it might well be misleading.

Correlation between mean diameter of receptacles and weight of flower heads. As the weighing of each individual head was impracticable it was considered advisable to ascertain for each category what degree of correlation existed between the mean diameters and the weights of the heads. Dr Wishart kindly determined the correlation coefficients for the whole range, they were:

1st week buttons *0·865*. 2nd week buttons *0·885*.
3rd week buttons *0·631*. 4th week buttons *0·621* closed showing petals *0·588*.
5th week buttons *0·861* closed showing petals 0·278 half open 0·319.
6th week closed showing petals *0·690*, half open *0·858*, three-quarters open *0·834*, fully open *0·564* (?).
7th week fully open 0·326 (11 pairs).
8½ weeks overblown *0·872*.

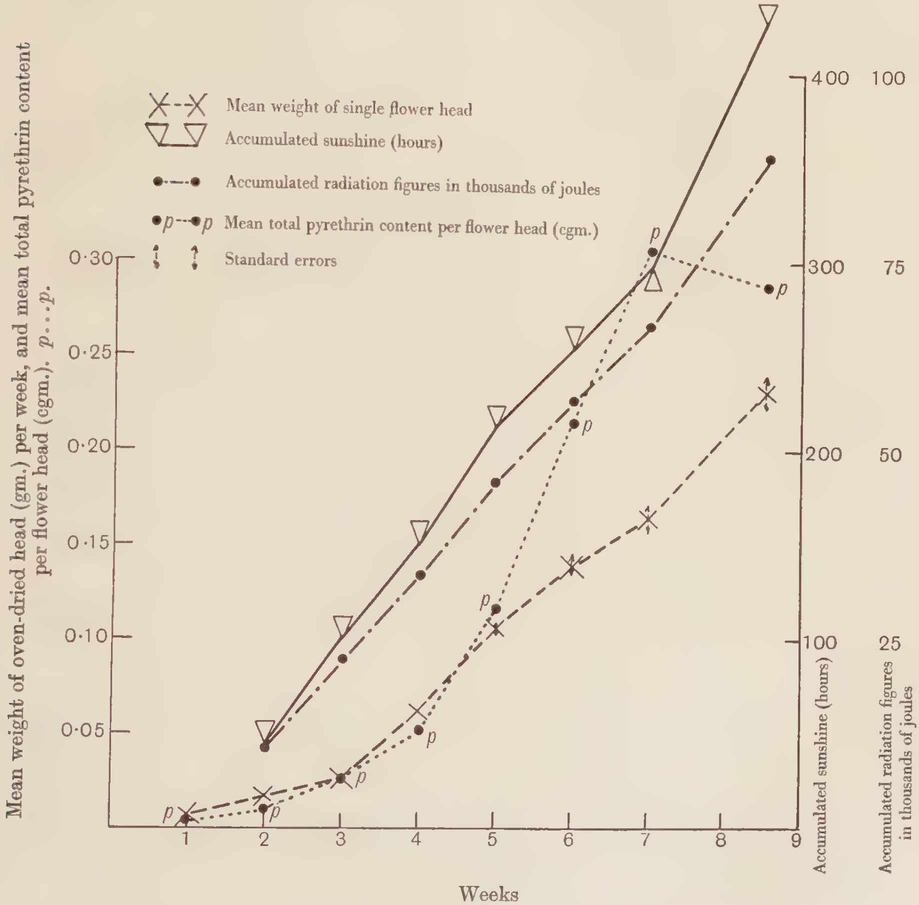
For twelve pairs of values figures greater than 0·576 and for eleven pairs of values greater than 0·602 indicate a significant correlation, they are represented in italics. Thus, there is no doubt that the correlations are significant over the major portion of the period, but begin to fail



Text-fig. 1. Growth rate of pyrethrum flowers and radiation figures.

when the flower opens out. It is probable that the mean weight more truly represents the average sizes of the flowers than the diameter of the receptacle in the later stages of growth, as some difficulty is experienced in measuring the latter values, but that the mean diameter of the receptacle does roughly indicate the range of sizes over the period.

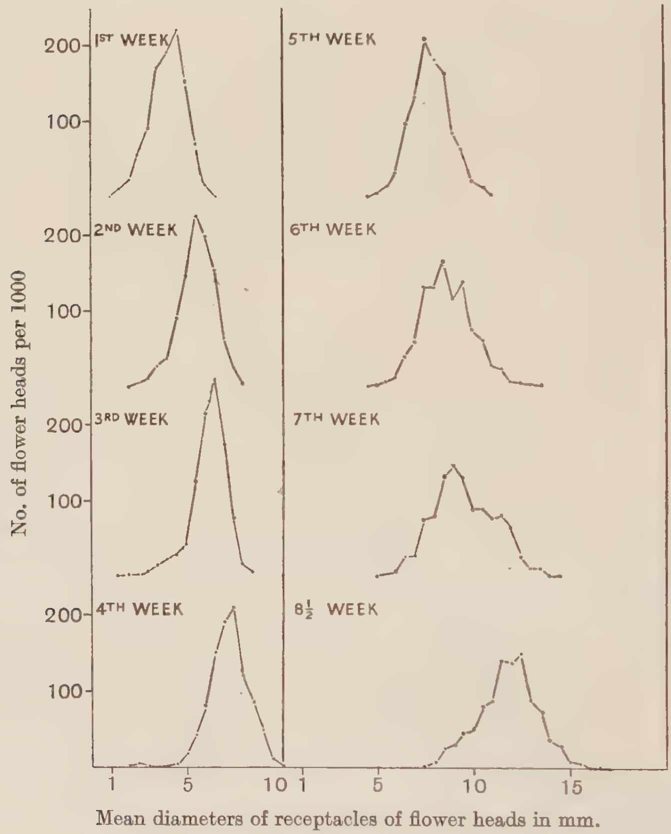
The distribution of mean sizes of the flower heads. Text-fig. 3 shows the distribution curves of the size of the flower heads as represented by the mean diameters of their receptacles. Each point represents the numbers of heads per thousand, placed in classes differing in diameter



Text-fig. 2. Growth of pyrethrum flowers, and radiation figures (accumulated).

from each other by approximately 0·5 mm. The modes of the curves, as would be expected, move to the right with time, but there is also a tendency for the curves to flatten out after the third week, indicating an increase in heterogeneity with respect to size, which would be expected in the fourth, fifth and sixth weeks, as the buds open out at different rates during these weeks, and there is some overlap in the sizes among

the different categories; it should, however, be noted that in the material in the seventh week there is a further slight increase in heterogeneity of size. In this case the flowers are nearly all open. The distribution curve for the overblown heads taken $8\frac{1}{2}$ weeks after the first crop is



Text-fig. 3. Distribution of mean sizes (diameters in mm.) of receptacles of flower heads.

significantly to the right of the others, due undoubtedly to pollination and the formation of achenes having given rise to a rapid increase in the size of the receptacles.

The percentage pyrethrin content. The mean percentage content of the pyrethrins both on the air- and oven-dried heads are given in Table VII.

Table VII.

Percentage of pyrethrin I on air-dried flower heads.

Week	1	2	3	4	5	6	7	8½
General mean	0·12	0·21	0·25	0·435	0·455	0·708	0·75	0·497
Mean by plants	—	—	—	0·441	0·480	0·672	0·700	0·504

Analysis of variance of weeks 4–8½.

	Degrees of freedom	Sum of squares	Mean square	½ $\log_e$	"z"
Dates	4	0·67246	0·16812	1·4110	0·8740
Blocks	11	0·61884	0·05626	0·8637	0·3267
Remainder	43	1·25872	0·02927	0·5370	—
Total	58	2·55002			

Effect due to date—significant.
Effect due to blocks—not quite significant.
Standard error estimation for single plant ±0·17.
Standard error estimation for twelve plants ±0·049.

Percentage content of pyrethrin I on oven-dried flower heads.

Week	1	2	3	4	5	6	7	8½
General mean	0·135	0·235	0·28	0·485	0·51	0·80	0·85	0·556
Mean by plants	—	—	—	0·493	0·536	0·757	0·795	0·561

Analysis of variance of weeks 4–8½.

	Degrees of freedom	Sum of squares	Mean square	½ $\log_e$	"z"
Dates	4	0·905108	0·226277	1·55959	0·57474
Blocks	11	0·788551	0·071686	0·98485	0·32700
Remainder	43	1·602772	0·037274	0·65785	—
Total	58	3·296431			

Effect due to date—significant but less than 1 per cent. point.
Effect due to blocks—not quite significant.
Standard error of estimation for single plant ±0·193.
Standard error of estimation for twelve plants ±0·056.

Percentage of pyrethrin II on air-dried flower heads.

Week	1	2	3	4	5	6	7	8½
General mean	0·24	0·38	0·576	0·644	0·525	0·656	0·89	0·643
Mean by plants	—	—	—	0·632	0·566	0·705	0·832	0·635

Analysis of variance of weeks 4–8½.

	Degrees of freedom	Sum of squares	Mean square	½ $\log_e$	"z"
Dates	4	0·49150	0·12288	1·2542	0·5963
Blocks	11	0·13644	0·01240	—	—
Remainder	43	1·60313	0·03728	0·6579	—
Total	58	2·23107			

Effect due to date—significant but less than 1 per cent. point.
Effect due to blocks—not significant.
Standard error of estimation for single plant ±0·193.
Standard error of estimation for twelve plants ±0·057.

Table VII (*cont.*).

Percentage of pyrethrin II on oven-dried flower heads.

Week	1	2	3	4	5	6	7	$8\frac{1}{2}$
General mean	0·27	0·425	0·63	0·718	0·59	0·74	1·00	0·686
Mean by plants	—	—	—	0·707	0·63	0·796	0·944	0·703

Analysis of variance of weeks 4–$8\frac{1}{2}$.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"
Dates	4	0·693275	0·173319	1·42627	0·48449
Blocks	11	0·169668	0·015424	0·21666	—
Remainder	43	2·828078	0·065769	0·94178	—
Total	58	3·691021			

Effect due to dates—significant approximately 5 per cent. point.
Effect due to blocks—not significant.
Standard error of estimation for a single plant $\pm 0{\cdot}256$.
Standard error of estimation for twelve plants $\pm 0{\cdot}074$.

Percentage of total pyrethrins on air-dried flower heads.

Week	1	2	3	4	5	6	7	$8\frac{1}{2}$
General mean	0·36	0·59	0·824	1·079	0·98	1·364	1·64	1·11
Mean by plants	—	—	—	1·073	1·045	1·376	1·532	1·139

Analysis of variance for weeks 4–$8\frac{1}{2}$.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"	Sum of products pyrethrin I and pyrethrin II	"r"
Dates	4	2·15987	0·53997	1·99447	1·03359	0·49795	0·86615
Blocks	11	1·14760	0·10433	1·17247	0·21159	0·19616	0·6751
Remainder	43	2·93805	0·06833	0·96088	—	0·03810	0·02682
Total	58	6·24552					

Effect due to date—significant.
Effect due to blocks—not significant.
Standard error of estimation for a single plant $\pm 0{\cdot}2614$.
Standard error of estimation for twelve plants $\pm 0{\cdot}075$.
The correlation of percentages of pyrethrin I and II for blocks—significant (from "r" test).
The correlation of percentages of pyrethrin I and II for dates—doubtfully significant.
The correlation of percentages of pyrethrin I and II for single plants—not significant.

Percentage of total pyrethrins on oven-dried heads.

Week	1	2	3	4	5	6	7	$8\frac{1}{2}$
General mean	0·405	0·66	0·91	1·203	1·09	1·54	1·85	1·24
Mean by plants	—	—	—	1·200	1·168	1·553	1·740	1·265

Table VII (*cont.*).

Analysis of variance for weeks 4–8½.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"	Sum of products pyrethrin I and pyrethrin II	"r"
Dates	4	2·993182	0·748295	1·00631	1·00178	0·697400	0·88040
Blocks	11	1·468745	0·133522	0·14452	0·13999	0·253263	0·69790
Remainder	43	4·542738	0·100950	0·00453	—	0·055944	0·02630
Total	58	9·004665					

Effect due to date—significant.
Effect due to blocks—not significant.
Standard error of estimation for a single plant $\pm 0{\cdot}317$.
Standard error of estimation for twelve plants $\pm 0{\cdot}0916$.
The correlation of percentage of pyrethrin I and II for blocks—significant (from "r" test).
The correlation of percentages of pyrethrin I and II for dates—just significant.
The correlation of percentages of pyrethrin I and II for single plants—not significant.

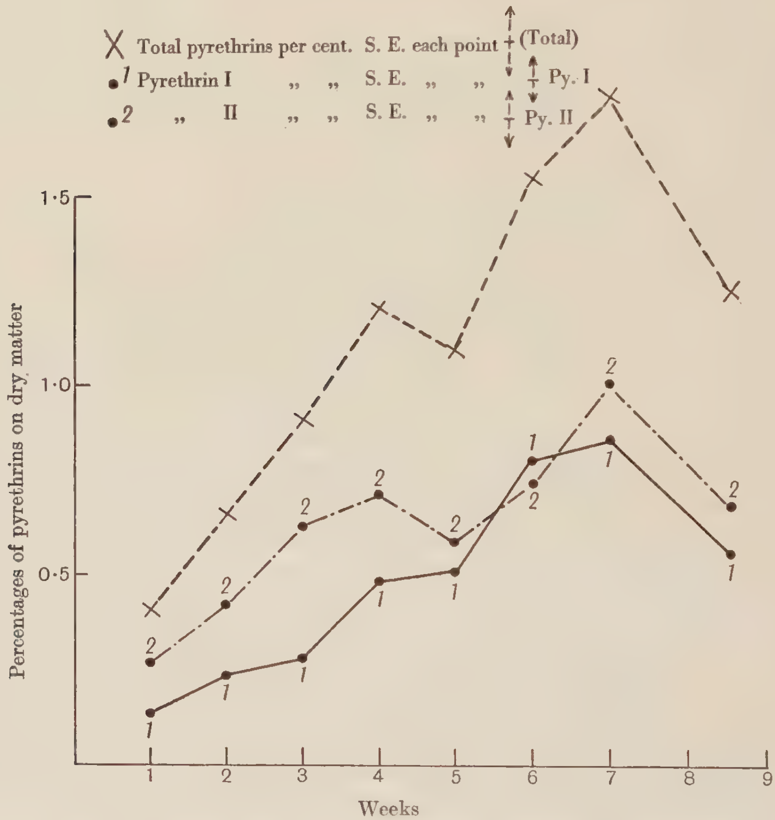
The application of the "z" test to the data in Table VII shows that the date upon which the flower heads were gathered had a significant bearing upon their percentage content of pyrethrin I and II, whether considered separately or together, and whether calculated to the air- or oven-dried material. It is true that only the crops taken on the last five occasions have been considered, when individual plants gave a large enough yield for separate analysis, but it is clear that the date effect in the cases of pyrethrin I and II taken separately could occur by chance not more frequently than between 1 in 100 and 1 in 20 times, and less than 1 in 100 times as in the case of the total pyrethrins. The effect is undoubtedly due to maturation. It was possible also that some unevenness in the distribution of the chemical or physical properties of the soil over the plot might have had some effect on the percentage content of the pyrethrins—it was found not to be significant, although significance is approached in the case of pyrethrin I. In addition, in Table VII the degree of correlation was sought for the way in which pyrethrin I and II vary together by means of the "r" test

$$r = \frac{\text{covariance (pyrethrin I . pyrethrin II)}}{\sqrt{\text{variance pyrethrin I} \times \text{variance pyrethrin II}}},$$

the covariance being equal to the sum of the products divided by the number of degrees of freedom. The results of the analysis show that there is a significant correlation for the blocks. For individual plants the correlation was not significant, owing probably to the heterogeneity of the material. The correlation for the dates of harvesting, that is, for maturity,

is just about significant, as the values are not likely to occur by chance more frequently than 1 in 20 times.

The general mean percentage figures are plotted against the dates in Text-fig. 4.



Text-fig. 4. Weekly averages pyrethrin content of flowers in percentages, on dry matter.

Table VII and Text-fig. 4 demonstrate that throughout most of the period the flower heads show a higher percentage of pyrethrin II than pyrethrin I, and that the curves follow a more or less parallel course. On the sixth week the mean value of pyrethrin I is higher than that of pyrethrin II—a result largely due to one plant harvested that week having a crop of flower heads with an exceptionally high percentage of pyrethrin I. After maturity, when pollination has taken place, the percentage of the pyrethrins falls.

The pyrethrin content per flower head. The mean contents of the pyrethrins in milligrammes per flower head are given, together with an analysis of variance in Table VIII. Owing to the change in the weight of the flowers with time affecting these values progressively, the standard errors of the means by plants for the last five weeks have been calculated separately instead of the general standard error estimated from the mean square of the remainder.

Table VIII.

Content of pyrethrin I in mg. per flower head.

Week	1	2	3	4	5	6	7	8½
General mean	0·01	0·039	0·077	0·25	0·54	1·11	1·40	1·29
Mean by plants	—	—	—	0·27	0·55	1·04	1·29	1·31
Standard error of mean	—	—	—	±0·037	±0·056	±0·10	±0·14	±0·11

Analysis of variance for weeks 4–8½.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"
Dates	4	10·423139	2·605785	2·78143	1·68077
Blocks	11	1·775095	0·161372	1·66277	0·56211
Remainder	43	3·889786	0·090460	1·10066	—
Total	58	16·088020			

Effect due to date—highly significant.
Effect due to blocks—significant (about 1 per cent. point).

Content of pyrethrin II in mg. per flower head.

Week	1	2	3	4	5	6	7	8½
General mean	0·02	0·07	0·18	0·37	0·62	1·03	1·66	1·59
Mean by plants	—	—	—	0·37	0·64	1·10	1·53	1·63
Standard error of mean	—	—	—	±0·034	±0·032	±0·072	±0·15	±0·125

Analysis of variance for weeks 4–8½.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"
Dates	4	14·314665	3·578666	2·94009	1·62472
Blocks	11	0·597962	0·054360	0·84652	—
Remainder	43	5·969552	0·138827	1·31537	—
Total	58	20·882179			

Effect due to date—highly significant.
Effect due to blocks—not significant.

Content of total pyrethrins in mg. per flower head.

Week	1	2	3	4	5	6	7	8½
General mean	0·03	0·11	0·26	0·62	1·16	2·14	3·06	2·88
Mean by plants	—	—	—	0·64	1·19	2·14	2·82	2·94
Standard error of mean	—	—	—	±0·05	±0·08	±0·13	±0·26	±0·17

Table VIII (*cont.*).

Analysis of variance for weeks 4–8½.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"	Sum of products	"r" correlation
Dates	4	48·897515	12·224379	2·40298	1·88570	12·079855	0·98893
Blocks	11	3·520391	0·320036	0·58157	0·06429	0·573667	0·5568
Remainder	43	12·099865	0·281392	0·51728	—	1·120263	0·2325
Total	58	64·517771					

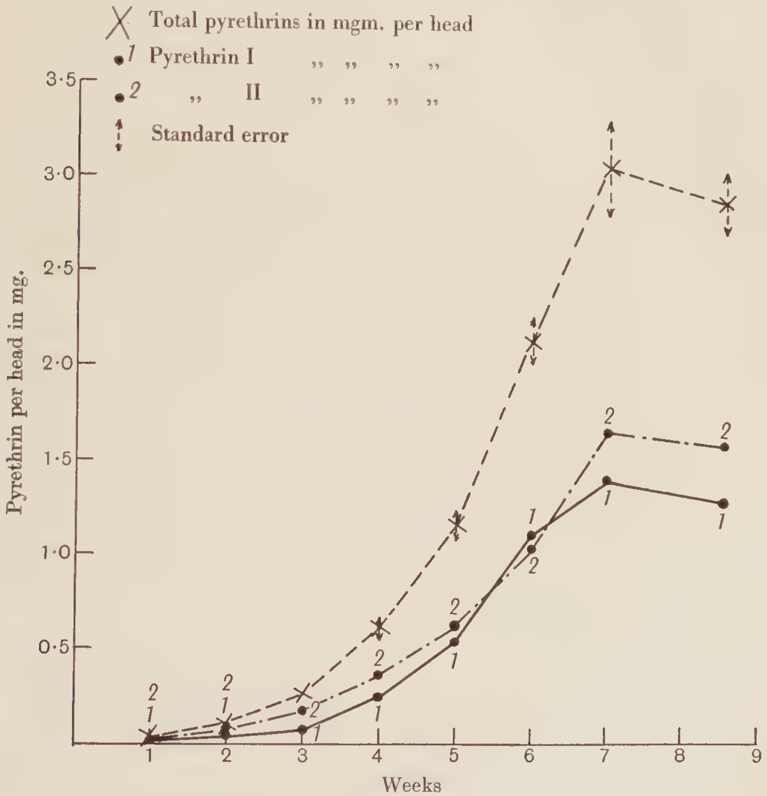
Effect due to date—highly significant.
Effect due to blocks—almost significant.
Correlation of contents of pyrethrin I and II per flower head for dates—significant.
Correlation of contents pyrethrin I and II per flower head for blocks—significant.
Correlation of contents pyrethrin I and II per flower head for individual plants—not significant.

Table VIII demonstrates the high significance of the date of harvesting upon the absolute yield of the pyrethrins per flower head whether considered separately or in combination, an effect largely due to the degree of maturation. There is also a close correlation with date for the relative amounts of pyrethrin I and II. The effect of the position in the beds (block effect) upon yield of pyrethrin per head only rises to significance in the case of pyrethrin I and has no significance in the case of the total pyrethrins. At this stage it is not possible to offer any explanation for this and too much stress should not be laid upon it. It is, however, important to notice that the degree with which the two active principles vary together is of significance for the various blocks, but not for individual plants, where it is probably neutralised by the general heterogeneity of the material. The data for the pyrethrin content per flower head are plotted against the dates in Text-fig. 5.

The close similarity of the three curves in Text-fig. 5 is a noticeable feature and brings out the close correlation of the pyrethrin content per flower head with the times of harvesting, shown in the foregoing analyses. The falls in the pyrethrin contents after pollination cannot be regarded as significant, being less than the standard error of the respective means; not only so, but had the means by plants been plotted instead of the general mean a slight but not significant rise in pyrethrin content would have been noted in this region. In Text-fig. 6, the data for the total pyrethrin content per head is plotted on the same diagram as the general mean percentage content of total pyrethrins and also the general average weight of the single flower head taken each week. In addition, there are given figures indicating the average degree of maturity of the flowers taken each week. In all the diagrams, wherever possible without con-

fusion, an attempt is made by means of double arrows to indicate the standard errors of the means.

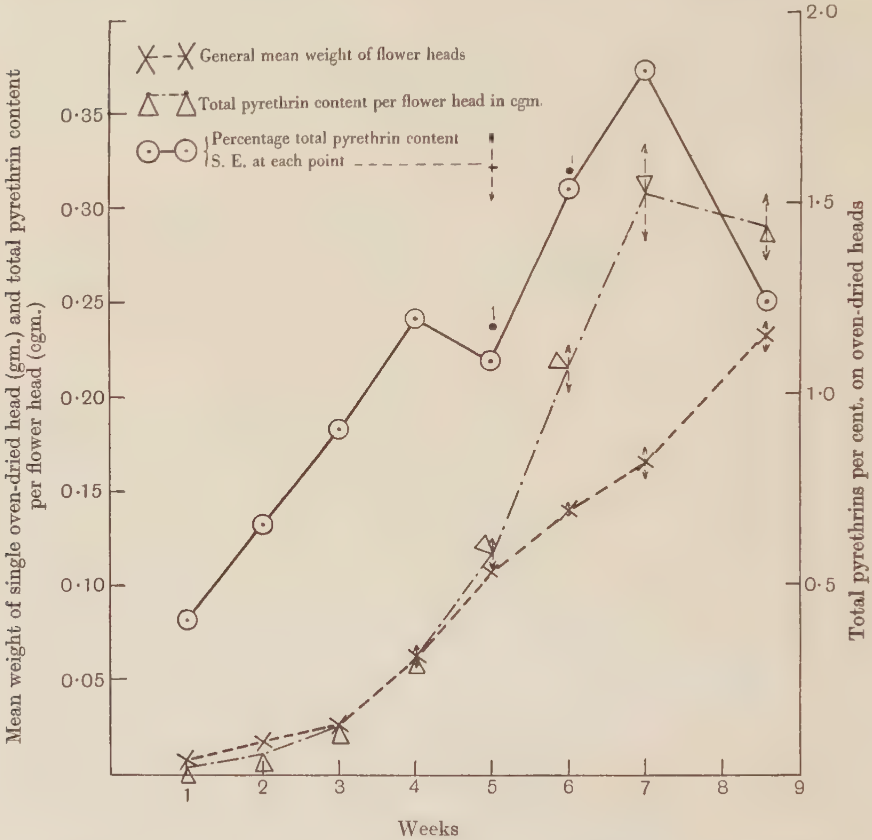
The points represented by circles indicate the general mean percentage content of total pyrethrins. It may have no significance but the values for weeks 1–4 and 5, 6 are such that a straight line could be drawn to fit them fairly closely. Their most noticeable feature is, however, the



Text-fig. 5. The pyrethrin content in mg. per flower head each week.

fall which takes place between the fourth and sixth week. This is partly due to the presence of an exceptional plant amongst the crop taken on the fifth week; nevertheless, if this value is neglected the general mean only rises to the value represented by the symbol ●′, and the break is still conspicuous. The general mean value for the percentage pyrethrin content for the fifth week is 1·09, the mean of the values for the fourth and sixth week 1·37. The difference between these values which represents the

actual fall in pyrethrin content per cent. is 0·26, with a standard error of $0{\cdot}09/\sqrt{2} = \pm\, 0{\cdot}08$. The decline is therefore significant. Reference to



Weeks	1	2	3	4	5	6	7	8½
	%	%	%	%	%	%	%	%
State of flowers	100 *B*	100 *B*	100 *B*	83·6 *B*	8·3 *B*	2·15 *B*	2·1 *B*	100 *O*
				16·4 *CP*	64·7 *CP*	16·55 *CP*	6·4 ½–¾	
					20·1 ½	12·4 ½	91·5 *F*	
					6·9 ¾–*F*	68·9 *F*		

B = buttons, *CP* = closed showing petals, ½ = half open, ¾ = three-quarters open, *F* = fully open, *O* = overblown.

Text-fig. 6. Pyrethrin content of flower heads for each week of growth.

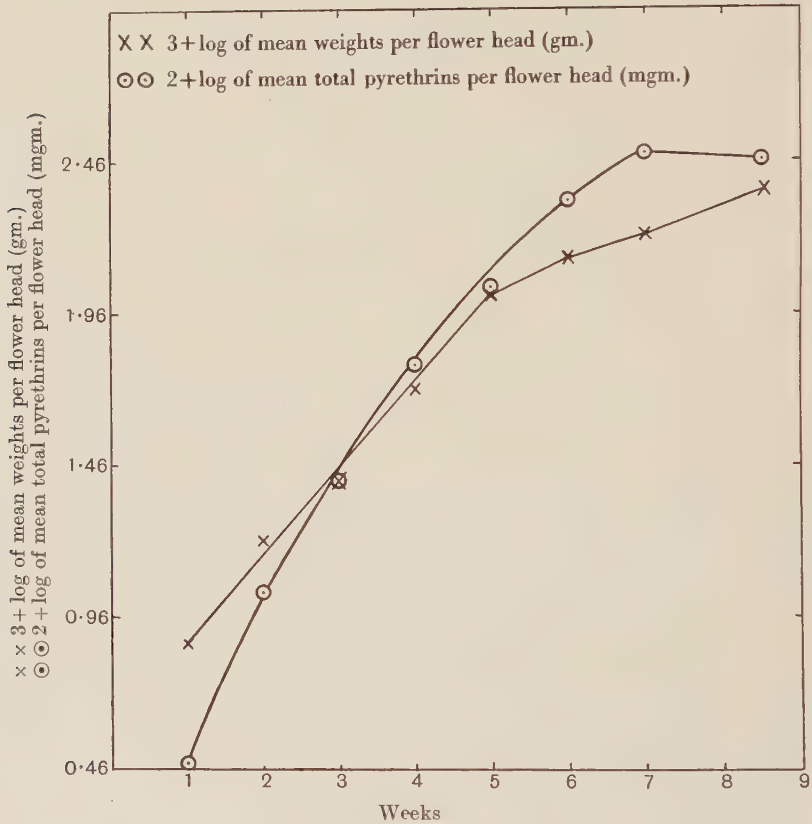
the curve representing the average weight of the single flower head each week shows, however, that the break in the percentage content falls at a period when growth has been most rapid, and it is obvious that the percentage pyrethrin content has not kept pace with the increase in

weight. The point for the percentage content on the sixth week is also slightly low, but in this case the elimination of one plant, which bore an exceptionally large number of heads slightly less rich in pyrethrin than the average for that week, would bring the general mean value more into line. Although these results may be due to some factor external to the flower, it may be significant that the ray florets contain very little of the active principles, and that during both of these periods the flowers were in the process of opening out, for between the fourth and fifth week, the flowers showing petals have risen from 16·4 per cent. to 91·7 per cent. and between weeks 5 and 6 the flowers, fully open, have risen from 6·9 to 68·9 per cent. The curve representing the general mean content of pyrethrin per flower head shows no break, and from this it is clear that the drop in percentage values does not in any way imply any loss of the pyrethrins in the flower head, but that under our conditions their increase has not been commensurate with the general development of the flower at this stage. Text-fig. 6 demonstrates the fall in the pyrethrin percentage between maturity and the over-blown condition. Reference to the growth curve shows that the increase in weight of the flower head at this stage is accentuated by pollination, and since fall in the absolute value of the pyrethrins after pollination is not significant, this increase in weight has apparently not been accompanied by any further synthesis of the active principles, although there has been no actual loss. It is evident, therefore, that the pyrethrin content reaches a maximum value when the flower is fully open, whether considered as percentages or as amounts per flower head.

It is of interest to ascertain the relative rates of increase of weight in the flower heads and of their content of pyrethrins. In Text-fig. 7 we have plotted against the dates the logarithms of the average weight of the single flower head each week and its general mean content of total pyrethrins.

The points showing the relative increase in weight of the flowers (represented in Text-fig. 7 by ×'s) from the earliest stages to near maturity fall close to a straight line drawn between these points. After the fifth week, when the maturation commences, the slope of the curve becomes progressively less steep until pollination occurs between the sixth and seventh week and causes a further accentuation of steepness. The points representing the logarithms of the weekly general means in pyrethrin content per flower head fall on or near a curve the slope of which declines in steepness progressively with time, indicating that in our experiment the relative rate of synthesis of the total pyrethrins has

fallen off with time. After the seventh week there is a break in the curve and the value for the overblown flowers after $8\frac{1}{2}$ weeks is not significantly different from that of mature flowers taken at the end of the seventh week. Thus the pyrethrins would appear to accumulate steadily in the flower head with time, although in our case at a progressively retarded relative rate, they more than keep pace with the



Text-fig. 7. Relative increases in weight of head and pyrethrin content.

increase in weight of the head, achieving a maximum content when the flowers are fully open. Although no loss in absolute amount occurs after pollination, the percentage values are lowered by the failure of the plant either to synthesise or translocate the active principles on the flower fading after fertilisation.

The pyrethrin content of flowers in different stages of maturity taken at the same time. Two plants harvested in the fifth and six weeks respec-

tively bore a sufficient number of flowers to make possible an analysis of the flowers in varying stages of openness. The data are set out in Table IX.

Table IX.

Analysis of flowers in different stages of maturity taken at the same time.

No. of lant	Time taken, week	Degree of maturity	No. of flower heads	Weight of oven-dried heads (gm.)	Pyrethrin I % on oven-dried heads	Pyrethrin I Mg. per head	Pyrethrin II % on oven-dried heads	Pyrethrin II Mg. per head	Total pyrethrins % on oven-dried heads	Total pyrethrins Mg. per head
12	5th	2·1 % buttons 97·9 % closed showing petals	142	12·329	0·25	0·22	0·34	0·30	0·59	0·52
12	5th	97·0 % half open 3·0 % fully open	168	21·745	0·36	0·47	0·42	0·54	0·78	1·01
1	6th	Closed showing petals	89	8·134	0·66	0·60	0·48	0·44	1·14	1·04
1	6th	Half open	68	7·898	0·68	0·79	0·55	0·64	1·23	1·43
1	6th	Three-quarters open	39	4·813	0·73	0·90	0·61	0·75	1·34	1·65
1	6th	Fully open	244	36·002	0·78	1·15	0·60	0·89	1·38	2·04

In the fifth week the heads fell mostly into the categories of "closed showing petals" and "half open" and in the sixth week they ranged from "closed showing petals" to "fully open." Although nothing at present is known of the degree of variation existing between flowers upon the same plant, the analyses indicate that this cannot be very large, but that with increasing maturity there is an increase in the content of pyrethrins. It is true that in our analyses between consecutive categories the increase in pyrethrin content is hardly outside the range of the experimental error, nevertheless the tendency is all in one direction and affords corroborative evidence that the pyrethrin content of the flowers is correlated with the degree of maturity.

Table X.

Contents of pyrethrins in mg. per plant.

Weeks	1	2	3	4	5	6	7	$8\frac{1}{2}$
Mean pyrethrin I in mg.	1·39	6·38	12·1	39·4	78·6	180·9	194	205
Standard error	—	—	—	±5·7	±12·2	±39·6	±35·5	±21·5
Mean pyrethrin II in mg.	2·78	11·5	28·1	58·5	90·6	167	230	254
Standard error	—	—	—	±9·7	±12·0	±18·0	±42·2	±26
Mean total pyrethrins in mg.	4·17	17·9	40·2	97·9	169	348	424	459
Standard error	—	—	—	±11·7	±23·4	±59·8	±75·6	±42

Table X (*cont.*).

Analysis of variance pyrethrin I.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"
Dates	4	272,854·08	68,213·52	2·11132	1·12843
Blocks	11	123,877·48	11,261·59	1·21062	0·22773
Remainder	43	307,041·79	7,140·51	0·98289	—
Total	58	703,773·35			

Analysis of variance pyrethrin II.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"
Dates	4	349,920·61	86,980·15	2·23284	1·32617
Blocks	11	123,885·38	11,262·31	1·21067	0·30400
Remainder	43	263,629·20	6,130·91	0·90667	—
Total	58	734,835·19			

In both cases the effect due to dates is clearly significant, but there is no block effect.

Analysis of variance total pyrethrins.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"	Sum of products	"r" (correlation)
Dates	4	1,214,005·90	303,501·48	1·70640	1·28251	296,615·6	0·96269
Blocks	11	470,093·83	42,735·80	0·72622	0·30233	111,165·5	0·89826
Remainder	43	1,003,818·50	23,344·62	0·42389	—	216,573·8	0·76121
Total	58	2,687,918·23					

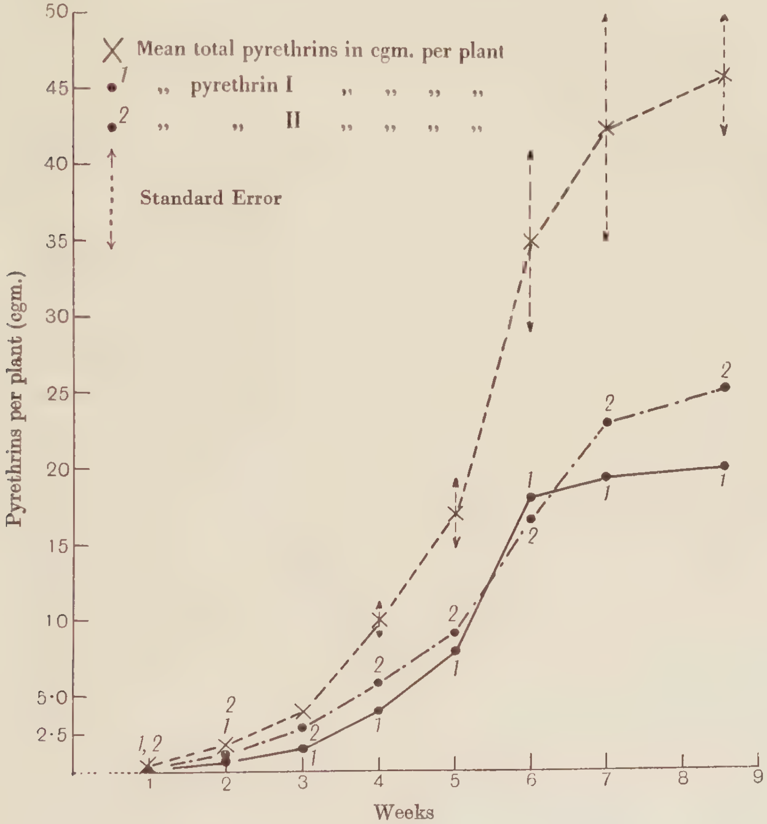
Effect due to date—clearly significant.
Effect due to blocks—not significant.
Correlation of contents of pyrethrins I and II per plant for dates—significant.
Correlation of contents of pyrethrins I and II per plant for blocks—significant.
Correlation of contents of pyrethrins I and II per plant for individual plants—significant.

The pyrethrin content per plant. From the point of view of the grower of pyrethrum the efficiency of the plants in the production of the pyrethrins is of importance. In Table X are set out the data and their analysis for the mean yield of pyrethrins per plant for the 12 plants from which the flowers were taken each week, together with the standard errors of the means for weeks 4–8½.

The results are plotted in Text-figs. 8 and 9.

It should be noted that the rises in the pyrethrin contents between the seventh and 8½ weeks are not significant as they are less than the standard error of the mean. The apparent increase in the pyrethrin content per plant after pollination and the withering of the flowers is thus largely due to chance or the heterogeneity of the material. In Text-fig. 9 the weight-growth curve takes the usual S-shape and the increases are significant up to the sixth week, where a marked flattening takes place; the mean weights per plant for the seventh week are not significantly

different from those of the sixth, subsequently a large rise in weight takes place due to the fertilisation of the flowers and the formation of seed.

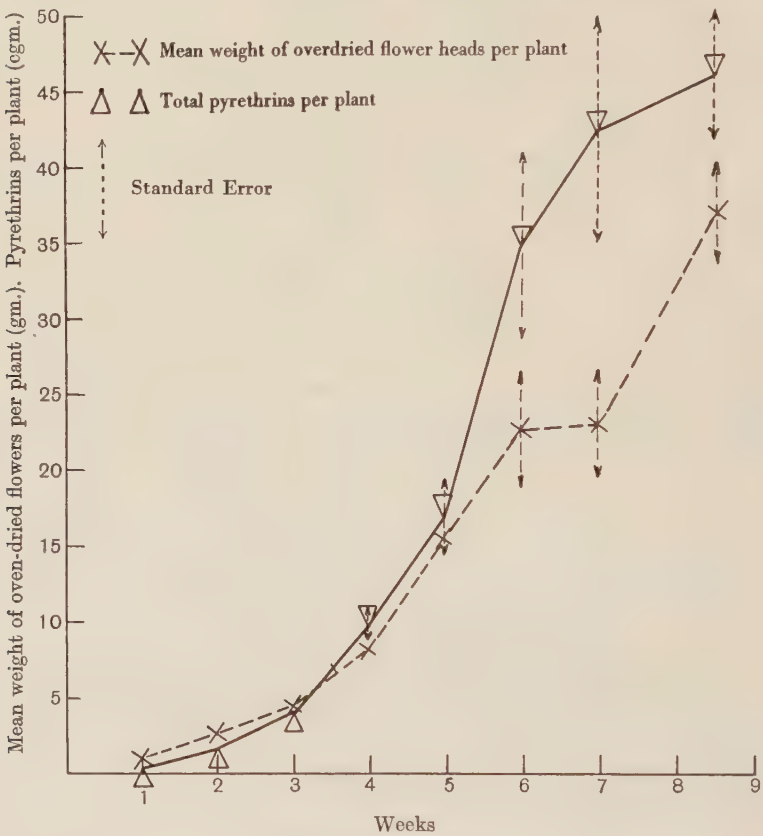


Text-fig. 8. Average pyrethrin content in cgm. per plant each week.

DISCUSSION AND CONCLUSIONS.

The toxicity to insects of pyrethrum flowers in various stages of growth has been examined by several investigators, and the view that "half-open" flowers are more potent than the "open" has been shown to be very doubtful. An examination of the problem, from the point of view of the pyrethrin content by Gnadinger and Corl (3), has demonstrated for *Pyrethrum roseum* a rise in the pyrethrin content (dry basis) of the combined active principles from the small unopened bud stage to the fully opened flower and for *C. cinerariaefolium* that the so-called

"closed" flowers have a lower pyrethrin content than the "open" flowers[1]. These conclusions are generally confirmed by our work. There has, however, been in the past some confusion in the terminology "fully open," "half open," "closed," owing to these descriptions having been applied to dry commercial samples. A number of growers have



Text-fig. 9. Yield of flower heads, and total pyrethrin per plant.

been led to take their crops at a comparatively early stage with the consequent loss of yield and pyrethrin content. These terms have a doubtful validity for the growing flower, *e.g.* Southall's *Materia Medica*

[1] Fryer, Tattersfield and Gimingham (*Ann. App. Biol.* 1928, xv, 423) had previously found by biological experiments that, weight for weight, the toxicity of "half open" was no greater than that of "fully open" flowers; and concluded that the correct time to harvest the flowers in practice is at the stage when the majority are "fully open."

describes the "closed" flowers as fully developed. In an article on "The pyrethrum industry in Japan" (5), the British Vice-Consul at Seoul states that the condition for picking is when the flower heads are 70 per cent. open. It is not clear whether this phrase describes the actual condition of the flower or of the crop as a whole. The writer, however, emphasises that loss of yield both of crop and active principles follows the practice of taking the harvest before the flower heads have partially opened. This is unquestionably true, but so long as the confusion of terms lasts, economic loss is likely to follow. We have attempted in our plates to give some visual actuality to our ascriptions, which are based on the flower before drying. There seems little doubt that the yield of pyrethrins as well as of flowers is at a maximum when they are in the fully opened state in the field. There is, however, a period during which the disk florets of the open inflorescence are themselves progressively opening, and it would appear that the extent to which this has happened determines the appearance of the flower when dry. No attempt has been made here to give the pyrethrin content for the flower heads in these different degrees of fully "openness." In some work carried out subsequently and published with J. T. Martin (*loc. cit.*), an attempt has been made to differentiate between them, and it appeared that with our material, up to the stage when all the disk florets were mature, there was a rise in pyrethrin content. From our plates, the flowers with more than one-half the total number of disk florets mature would in the dry state appear to warrant the description of being in the fully opened stage, in that the ray florets dry outwards and backwards, whereas those flowers in which the disk florets are only open to an extent of approximately half this number would apparently be regarded as "half open" when dry. Despite the slight superiority of the former it would be impossible in practice to select a large crop just at the optimum stage, and other countervailing factors might render it advisable despite some loss of weight to take the flowers somewhat earlier—thus the controversy over the respective values of the two categories might well become very artificial. It is evident, however, that for the grower a diminished yield both of flowers and active principles would result from taking flowers before the ray florets were well expanded.

Our plot, although of restricted size, showed considerable heterogeneity among the plants both in yield and in the pyrethrin content of the flowers. On certain plants flowers were borne which had in an early stage of development a higher pyrethrin content per cent. than others at a later stage of development. It is clear, since a wide heterogeneity in

this respect is likely to occur in any single crop and to be accentuated when comparisons are made between different crops grown under varying conditions as to climate and cultivation, that selection other than by direct analysis for the pyrethrin content or by biological test is to be deprecated.

In our view no useful purpose is served by taking the flowers to the overblown stage; in our material the increase in yield was largely neutralised by the fall in the percentage content of the pyrethrins, although its absolute amount per flower head suffered little change. Whether this were due to some partial loss of the achenes in harvesting it is not possible to say, but it appears improbable; since, however, in the overblown state the achenes[1] can readily be lost, the inadvisability of prolonging growth to this stage is obvious.

An attempt has been made by a statistical analysis of the data to show whether certain of the correlations examined were significant. The heterogeneity of the material rendered this imperative. The standard errors of the mean were where possible determined from the analysis of variance; this gives a more accurate estimation applicable to the whole table examined. Where, however, the errors progressively changed with time they were determined for the weekly values when data were available. In the main, however, the effects of two factors have been examined, that due to date and that due to the position of the plant in the bed. Although on examination it was ascertained that there was a significant variation in the maturity of flowers taken from different plants on the same date, on the average, the date of harvesting is a measure of the degree of maturation. The position of a plant in a bed may be one of considerable importance, for rarely, if ever, do the factors, soil or meteorological, have an equal effect on plant growth over the whole of even a small bed. The policy adopted of randomising the plants over blocks, the sum of which make up the plot, enables one to compute the significance of the effect due to the position. An analysis has shown that the effect due to date has been significant in the following cases—the weight yield of the flower heads and their mean weight, the moisture content, the percentage content of pyrethrin I, pyrethrin II and total pyrethrins both on air- and oven-dried heads, the content per flower head and per plant of the pyrethrins, both separately and together, and *not* significant in the case of the mean number of flower heads per plant. The effect due to position (block effect) was significant in the cases of

[1] Gnadinger and Corl have shown the achenes to contain 90 per cent. of the active principles (3).

the mean number of flower heads per plant, and the content per flower head of pyrethrin I and nearly so for the total pyrethrins per head; there was no significant effect in other cases.

The way in which the two pyrethrins vary together was also examined, the correlation for dates between the contents of pyrethrin I and II was significant, whether expressed in percentages, parts per flower head or parts per plant. When expressed in percentages, the correlation of the content of pyrethrin I and II for the blocks was significant, but not for individual plants, when expressed in parts per flower head the correlation was nearly significant for blocks but not for individual plants. The material was too variable in character to show an individual correlation for separate plants. When, however, the values were expressed in parts per plant a significant correlation was found for both blocks and individuals.

The data accumulated enable one to conclude for the material examined that there is a quantitative development of the active principles in the flower heads of pyrethrum from the small bud stage up to the time of maturity of the flowers. The synthesis of the active principles more than keeps pace with the increase in weight of the heads, and although fluctuations may take place at periods when rate of synthesis is not commensurate for a time with the increase in weight, the content of the pyrethrins both relatively and absolutely rises to a maximum at the maturity of the flowers. In our experiment the mean percentage content fell after fertilisation and the development of seed, but the amount per flower head remained constant.

Despite the rather high heterogeneity of our material, the average weight of the flower heads up to maturity followed an S-shaped curve usually associated with growth rates; after pollination, however, a rapid increase in weight was observed as seed developed. The curve for the mean content of pyrethrin in parts per head was also of a sigmoid type though less clearly defined. The relative mean weekly increases in weight of flower head were approximately linear in their course for several weeks—but as the flowers approached maturity the slope of the curve declined until fertilisation caused a further accentuation in steepness. While, however, the weight added to the flower head each week was roughly proportional to the weight already accumulated, the pyrethrin content in parts per head follows a course which indicated the relative rate of increase to be slowly declining. This may be due to lack of homogeneity in the flowers, but it is noteworthy that no abrupt break appears in the logarithmic curve until after maturity, at which point further synthesis of the active principles ceased altogether.

It may perhaps be worth notice that two varieties of flowers were observed on the bed, one having the usual appearance, and the other extremely small petals. The factor making for short petal was a genetical one, but so far has not been observed to be linked with high or low pyrethrin content.

Summary.

1. An account is given of the examination of the flowers of pyrethrum plants (*C. cinerariaefolium*) grown upon a bed in Harpenden. The plants were divided into blocks and randomised, the flowers being harvested from a dozen plants each week over a period of $8\frac{1}{2}$ weeks, the flower heads ranged from the small bud stage in the first week to the over-blown stage in the last week. The categories into which the flowers were divided to indicate maturity are defined and illustrated.

2. The yield in numbers and weight of heads per plant, the diameters of the receptacles and the content of pyrethrin I and II were determined. There was a considerable amount of variation in all these factors in the flowers from different plants.

3. A statistical analysis showed in this experiment:

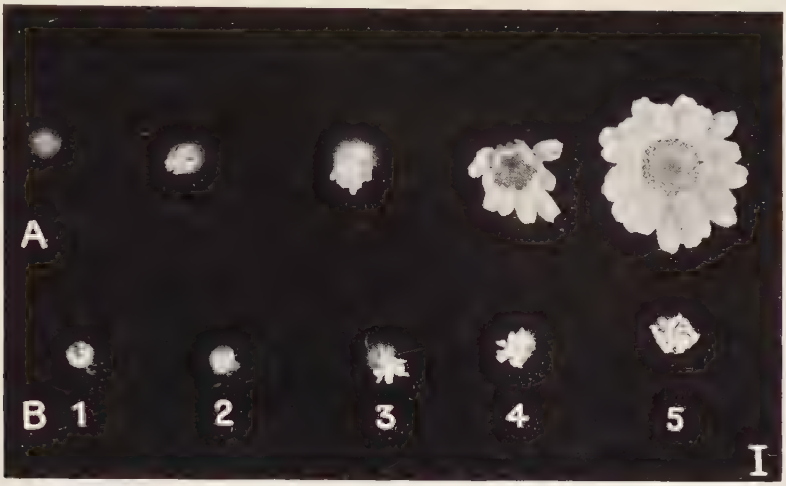
(*a*) That there was no significant variation in the numbers of the flowers with time, but that position of the plant in the bed had a significant effect.

(*b*) That the time of harvesting had a significant effect upon the content of the pyrethrins whether taken separately or together and whether expressed in percentages, parts per flower head or parts per plant. The effect due to position was only definitely significant in the case of pyrethrin I when expressed in parts per flower head.

(*c*) That there was on the different dates a significant correlation between the contents of pyrethrin I and II expressed in parts per flower head or plant.

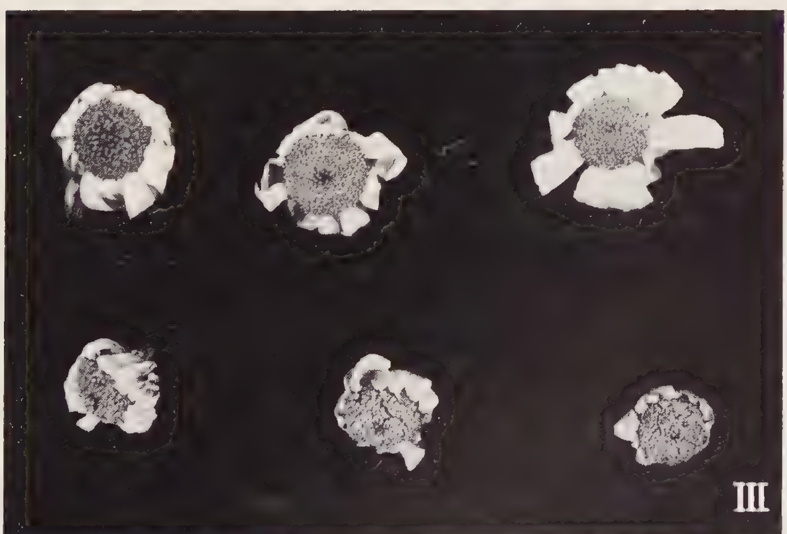
(*d*) That there was, for the blocks of material examined, a significant correlation between the contents of pyrethrin I and II, whether expressed in percentages, parts per head or parts per plant, but that owing to the heterogeneity of the material the correlation was only significant for individual plants when the pyrethrins were expressed in parts per plant.

4. The data in this experiment indicate that for the material examined, there is a quantitative development of the active principles in the flower heads from the small bud stage up to the time of maturity of the flowers, which more than keeps pace on the whole with the increase in weight of the flowers. Thus the content of pyrethrins, both relatively



As harvested

After drying



TATTERSFIELD.—PYRETHRUM FLOWERS (pp. 602–635).

and absolutely, rises to a maximum at the maturity of the flowers. The mean percentage content of pyrethrins fell after pollination and the fading of the flowers; this corresponds with the rapid increase in weight of the heads on the formation of seed. There would thus appear to be a loss, which might be serious, both in percentage content of active principles and in yield of flowers if harvested before being fully open. There seems to be no useful purpose served in leaving the flowers to the overblown condition.

I wish to express my great indebtedness to Dr R. A. Fisher and Dr J. Wishart for carrying out the greater part of the statistical analyses incorporated in this paper and for much helpful advice. I am also indebted to Mr J. T. Martin for checking many of the analyses by an alternative method.

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EXPLANATION OF PLATE XLIV.

Fig. 1 shows the categories into which the flower heads were divided for purposes of classification. Series A shows appearance just after harvesting, B after air-drying; No. 1 shows the closed bud or button stage; Nos. 2 and 3 closed bud showing petals. No. 4 half-open flowers; No. 5 fully open flowers.

Fig. 2 shows a range of fully open flowers. Series A gives appearance just after harvesting. Series B after air-drying. In flower No. 0 no disk florets are open; No. 1 has the first circle of disk florets open; No. 2 the first and second circles are open; No. 3 all are open to the third circle; No. 4 all are open to the fourth circle; No. 5 all are open except a few florets in the centre. When about half the number of circles of disk florets have opened the ray florets have dried outwards and backwards.

Fig. 3 represents flowers in the completely overblown stage both before and after drying.

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OBITUARY

MAJOR T. F. CHIPP, M.C., D.Sc., Ph.D.

General and Botanical Secretary of the Association of Economic Biologists, 1927.

Thomas Ford Chipp was one of those whose kindly spirit and interest in their fellows awaken feelings of friendliness in all with whom they have to do; his unexpected death at Kew on June 28th, 1931, constitutes a real loss to those who knew him.

He was born in 1886 at Gloucester and entered the Royal Botanic Gardens, Kew, in 1906 as a student gardener. Soon after his appointment he began to read for a degree at Birkbeck College. He obtained honours in botany in 1909 and was appointed demonstrator in the botanical department in the same year.

It was that autumn that I went to Birkbeck as head of the department. I remember him as a big, out-of-doors youngster, always ready to help, and as an ardent territorial who, as soon as his academic duties were over, would hurry to Richmond for a strenuous evening's drill.

In 1910 he was appointed Assistant Conservator of Forests on the Gold Coast and left for a year of preliminary study in Germany and the Federated Malay States before proceeding to his post.

In 1914 he accepted the Assistant Directorship of the celebrated Botanic Gardens at Singapore and was in England on his way to his new duties when war was declared. He obtained permission to rejoin the 8th Middlesex Regiment and served with distinction, gaining the Military Cross and being promoted to the rank of Major.

On demobilisation he proceeded at last to the Straits Settlements, but two years later a vacancy occurred on the Gold Coast, and he went back to that region as Deputy Conservator of Forests, returning to England in 1922 on his appointment as Assistant Director of the Royal Botanic Gardens, Kew. His work on the Gold Coast bore fruit in various publications and especially in his share of the volume on *Aims and Methods in the Study of Vegetation*, which he edited jointly with Prof. A. G. Tansley. It brought him also, in 1927, the degree of Doctor of Science in the University of London. For this he was registered at his old college, so that, after nearly twenty years, he was once more on the

staff at Kew and was nominally at least again a student at Birkbeck. It was pleasant to have this excuse for many interesting talks about his overseas experience, and there is no doubt that his early work at Kew rendered him particularly fitted to enter into the activities of the gardeners and to help them with sympathy and advice.

Chipp was essentially an outdoor botanist, his special subjects were forestry and ecology. He was interested in plants and understood them, but he was also a sound administrator, and many who do not know his official work will remember how pleasantly and efficiently he fulfilled his duties as joint secretary of the International Botanical Congress in 1930. He is one of those who can ill be spared.

H. C. I. G.-V.

REVIEWS

Die Forstinsekten Mitteleuropas. Dritter Band. Lepidopteroidea. Von K. ESCHERICH. Pp. 825 with 605 figs. and 14 coloured plates. Berlin: Paul Parey, 1931. 57 M.

The last decade or so witnessed a remarkable development in the fundamental ideas of economic entomology. Descriptions of observations on life histories, sometimes supplemented by experimental work, but never venturing beyond pure empiricism, are giving way to critical studies on insects as component elements in the respective communities and to a thorough analysis of the environment in relation to the physiology of the insect. The main practical problem before economic entomologists of not long ago was to find a vulnerable spot in the life history and to devise a method of attack on the pest. At present this is not considered sufficient, and it becomes an ambition of entomologists to be in the position not only to control the insect after it appeared as a pest, but to foresee an outbreak and to forestall it. This study of the course of outbreaks is essentially parallel to studies on epidemics of human and animal diseases, and the name *epidemiology of insect pests* is probably one which most fittingly defines the contents of economic entomology. Epidemiological work of this kind developed mainly in Germany and particularly with regard to certain forest insects, and Prof. K. Escherich and his numerous pupils achieved notable results, deserving the close attention of every economic entomologist.

The present book has a much more general interest than can be judged from its title. Indeed, its chief interest is in the introductory chapters dealing with the whole problem of the epidemiology of insect pests in a very clear and concise manner. Again, the chapters on *Bupalus piniarius* L. and especially that on *Panolis flammea* L. represent masterly accounts of an insect pest treated from an epidemiological point of view, and can justly be regarded as classical examples of epidemiological studies. The value of these studies for every economic biologist is obvious, since more or less the same methods, and indeed actually the same general ideas, would be applicable (and are applied) to studies on plant diseases, on fluctuations in numbers of animals, etc.

A forest entomologist will find in the book a mine of information on his subject. Detailed descriptions of all the more important, and many secondary, pests of forest trees are given and accompanied by copious illustrations of their various stages, and typical injuries. Useful keys to adult insects, their larvae and pupae and good bibliographies on each family will be found of great value. All illustrations, particularly the coloured plates of, so called, Microlepidoptera, are very well reproduced.

It is to be hoped that the title of the book will not prevent it from becoming known to, and appreciated by, all economic entomologists, and by biologists generally. The latter will be interested to find that economic entomology contains some matter of exceptional value for the understanding of the dynamics of animal life in nature, and particularly of the fluctuations in numbers on which the whole problem of the mechanism of evolution is largely based.

B. P. UVAROV.

Connecting Laws in Animal Morphology. By HANS PRZIBRAM. Pp. 62, with 8 plates comprising 23 figs. University of London Press Ltd., 1931. Price 4*s*. 6*d*. net.

Prof. Przibram's small book consists of four lectures delivered at the University of London in March, 1929. The main subject of these lectures is the importance of experimental morphology in relation to biological theory. Of recent years the experi-

mental method has made rapid growth in zoology and in this field of inquiry Prof. Przibram's name stands pre-eminent. He expresses his views and aims on p. 14 where he says: "I would refer to Physics and Chemistry as brilliant examples of what may be achieved in the way of unravelling the laws of nature by quantitative experiment and mathematical formulation based thereon. It is my firm conviction that we must proceed on the same lines if we wish Biology to develop into an exact science."

In discussing his subject Prof. Przibram deals with Organisation, Growth, Symmetry and Modification. The problems bear on the increase of Differentiation, of Organic Mass, of Growing Points and of Metabolic Rate, and the connecting laws between them. Each group of phenomena is illustrated from a different group of the animal kingdom—in the first crustaceans, in the second insects, in the third amphibians and in the fourth mammals. The lectures are written in an elementary style involving easily comprehensible methods and simple technique. Since this book treats of a subject outside the scope covered by the *Annals of Applied Biology* we are only able to make brief reference to it.

A. D. IMMS.

Industrial Microbiology. The Utilisation of Bacteria, Yeasts and Molds in Industrial Processes. By H. F. SMYTH and W. L. OBOLD. Pp. x + 313, with 3 plates and 11 text-figures. London: Baillière, Tindall and Cox, 1930. 27*s*. net.

This book attempts to fill a distinct gap in the literature of microbiology. Any single treatise in this comprehensive field must contain sharply selected material and in the present case the selection from widely scattered sources has been carried out with a view to its bearing on industrial practice, and the data are presented only in their technological relationships. Emphasis is laid particularly on the type and source of the organisms involved and the methods of controlling their functioning in large-scale operations. The book is in fact "dedicated to a more complete use of the microbiological processes in industry."

The text is conveniently planned. Following a preface and general introduction there are twelve sections each consisting of a number of chapters. Each section deals with a related group of problems which are as follows: I, Production of carboxylic acids; II, Production of alcohols and ketones; III, Complex nitrogenous materials; IV, Carbohydrate materials; V, Fats and oils; VI, Miscellaneous processes; VII, Microbial thermogenesis; VIII, Microbial food preparation; IX, Hydrocarbons; X, Commercial enzyme production; XI, Biological processes in industry; XII, Bacteriological survey. The individual chapters dealing with the particular problems are often extremely short—chapter VIII is only one page and chapter X only two pages—and each is followed by a bibliography giving references to industrial papers and patents.

There are a number of text-figures and tables and three unnecessary plates. There are also more misprints than in any book that I have read for years. For example in a table of seventeen lines containing the names of bacteria and fungi on pp. 150–1 the following misprints occur: *Sclerotina cinera, Mucor racemos, Rhizopus triciti, Botrytis cinera* and *Pennicillium*. The authors seem to be weaker in their spelling of the names of fungi than of bacteria, *e.g.* (p. 30) *Penicillum*, (p. 36) *Aspergillis*, (p. 44) *Monilla*, (p. 128) *Aspergillus wenti*, (p. 147) *Dermatia*, (p. 217) *Aspergillus tarnarii*, (p. 223) *Saccharomycetes cervisiae*, (p. 236) *Cladisporium*, (p. 304) *Monila, Sterigmatocystus* (p. 309), *Citromycetes*, (p. 312) *Saccharomyces cerevesiae*. A similar list could easily be constructed for author's names in the spelling of which Prof. Smyth and Obold show a light-hearted spontaneity. Further the book is rather badly written; *e.g.* the first paragraph on p. 66 ends as follows: "These organisms fermented lactic acid or calcium lactate to form proprionic and acetic acid, carbon dioxide and water. The free carbon dioxide being responsible for the holes in the cheese. They gave the

following formula." Sentences are often badly constructed or incomplete and occasionally very ambiguous and the authors sometimes split their infinitives in a most astonishing way. Sentences such as the following, p. 167, are not really uncommon: "If commercial yields would be the same as laboratory yields it is possible to produce 316 to 950 pounds of lactic acid per ton of sawdust, dry weight depending upon whether the sulphuric process of hydrochloric process is used. The sulphuric process yields less (see Table II)." Occasionally one is brought up very abruptly, *e.g.* "Coles (24) reports that only soil organisms can attack pectin," a sentence that probably no one would be more surprised to read than Coles.

The book is useful since there is no other covering the same field but, if a second edition is called for, it is to be hoped that the authors will take the opportunity of rewriting it.

WILLIAM B. BRIERLEY.

CAMBRIDGE: PRINTED BY W. LEWIS, M.A., AT THE UNIVERSITY PRESS